*Proc. Natl. Acad. Sci. USA*
Vol. 87, pp. 3792–3796, May 1990
Medical Sciences

# Changes in the sialylation and sulfation of secreted thyrotropin in congenital hypothyroidism

(glycosylation/pituitary)

PETER W. GYVES, NEIL GESUNDHEIT, N. RAO THOTAKURA, BETHEL S. STANNARD, G. STEPHEN DECHERNEY, AND BRUCE D. WEINTRAUB*

Molecular, Cellular and Nutritional Endocrinology Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Building 10, Room 8D14, Bethesda, MD 20892

*Communicated by Roy Hertz, February 6, 1990 (received for review November 30, 1989)*

ABSTRACT **We have examined the oligosaccharide structure of secreted thyrotropin (TSH) in perinatal and mature rats with congenital primary hypothyroidism. Rat pituitaries from euthyroid control animals and those rendered hypothyroid by methimazole treatment were incubated with [$^3$H]glucosamine *in vitro*. Secreted TSH was purified, and oligosaccharides were enzymatically released and characterized by anion-exchange HPLC. In perinatal hypothyroid animals compared with control animals, oligosaccharides from TSH $\alpha$ and $\beta$ subunits contained more species with three or more negative charges. Moreover, perinatal hypothyroid animals demonstrated a dramatic increase in the ratio of sialylated to sulfated species within oligosaccharides of the same negative charge (2.9- to 7.4-fold increase for TSH-$\alpha$; 15.1- to 25.5-fold increase for TSH-$\beta$). In mature hypothyroid 9-week-old animals compared with control animals, changes were less pronounced, suggesting that endocrine regulation of oligosaccharide structure is dependent upon the maturational state of the animal. These changes were specific for TSH because glycosylation of free $\alpha$ subunit (synthesized by the thyrotroph and gonadotroph) and of total glycoproteins was minimally altered by hypothyroidism. Together, these data provide direct evidence and characterization of specific changes in the structure of a secreted pituitary glycoprotein hormone occurring as a result of *in vivo* endocrine alterations during early development. Moreover, they provide a potential structural basis to explain the delayed clearance of both TSH and the gonadotropins with end-organ deficiency, which may have important implications for the *in vivo* biological activities of these hormones. Specifically, such posttranslational changes may be an important adaptive response to prevent the consequences of endocrine deficiency during early development.**

Thyrotropin (TSH) is a glycoprotein hormone containing an $\alpha$ subunit with two sites of asparagine-linked glycosylation and a $\beta$ subunit with one such site (1). Oligosaccharides on TSH play an important role in directing subunit combination, protecting the molecule from intracellular proteolysis, and influencing the metabolic clearance rate and biological activity of the circulating hormone (2–6).

In humans and experimental animals with intact hypothalamic–pituitary regulation, serum TSH levels are elevated in primary hypothyroidism (7) at least in part because of increased TSH subunit gene transcription (8–11). Moreover, measurement of TSH is used either as a primary or confirmatory screening test to detect congenital primary hypothyroidism, a preventable cause of mental retardation in children (12). However, it is not known whether structural alterations occur in TSH in congenital primary hypothyroidism or how the absence of thyroid hormone during prenatal development and early life affects the subsequent integration of the hypothalamic–pituitary–thyroid axis. The rat has proven to be an ideal animal to study TSH structure and regulation because maturation of the hypothalamic–pituitary–thyroid axis in this species is not complete until postnatal week 2 (13–15).

We have demonstrated (16, 17) that important changes in the glycosylation of secreted TSH occur in parallel with neuroendocrine maturation in the rat; these studies have shown that mature animals secrete TSH containing more negatively charged species with increased sialylation, decreased sulfation, and proportionately less binding to Con A than prenatal animals. In the present experiments, we compared oligosaccharides directly from secreted TSH $\alpha$- and $\beta$-subunit oligosaccharides from perinatal and mature rats with congenital primary hypothyroidism to those from control animals. We demonstrate that as little as 1 week of methimazole administration to pregnant rats, which blocks thyroid hormone biosynthesis, results in perinatal offspring with an increase in the percentage of negatively charged TSH subunit oligosaccharides compared with control animals. Moreover, TSH from these hypothyroid animals contained a dramatic increase in the relative sialylation compared with sulfation of species containing both one and two negative charges. These changes are less dramatic in mature hypothyroid animals than in control animals, suggesting that their magnitude is dependent upon the maturational state of the animal.

## MATERIALS AND METHODS

**Experimental Protocol.** Experiments were performed as described (16–18). Timed pregnant Sprague–Dawley rats were treated with 0.05% 2-mercapto-1-methylimidazole (methimazole; Aldrich Chem, Metuchen, NJ) in drinking water from day 16 gestation to the time of the experiment. Mature animals were maintained on 0.05% methimazole-containing drinking water after weaning. Control animals were obtained from similarly timed pregnant rats who received drinking water without methimazole. Control animals subsequently received drinking water without methimazole after weaning. Each sample from perinatal animals contained 10 pituitaries that were obtained from litters of these pregnant rats on either the last day of gestation (gestation day 23) or the day of birth. Samples from mature (8 week old) animals contained one pituitary. Thus, perinatal animals had 1 week of fetal hypothyroidism, while mature animals had 1 week of fetal and 8 weeks of postnatal hypothyoidism. All samples were incubated in Dulbecco's modified Eagle's medium containing 500 $\mu$Ci (1 $\mu$Ci = 37 kBq) per ml of [$^3$H]glucosamine for 24 hr. Incubations were ended by separating and freezing both pituitaries and media.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TSH, thyrotropin; N species, sialylated oligosaccharides; S species, sulfated oligosaccharides.

*To whom reprint requests should be addressed.

**Product Characterization.** Immunoprecipitation of TSH from medium was performed as described (16–18). Free $\alpha$ subunit was then immunoprecipitated, and total proteins were acid-precipitated in a sequential manner (19). TSH subunits were separated by sodium dodecyl sulfate (SDS)/15% polyacrylamide gel electrophoresis by the method of Laemmli (20). Subunits were eluted from the gel by shaking in a solution of 0.1 M ammonium bicarbonate/0.1% SDS at 25°C for 48 hr. Eluents were concentrated and desalted in Centricon-10 microconcentration chambers (Amicon). TSH subunits, free $\alpha$ subunit, and total proteins were then treated with endo-$\beta$-*N*-acetylglucosaminidase F/peptide *N*-glycosidase F (Endo F; Boehringer Mannheim; 0.5 units per sample) in buffer at 37°C for 18 hr (17). Released oligosaccharides were separated from the TSH apoprotein with Sep-Pak $C_{18}$ cartridges (Waters). Aliquots of TSH subunit oligosaccharides were also incubated with *Clostridium perfringens* neuraminidase (Sigma) in buffer at 37°C for 3 hr (17). Anion-exchange HPLC of released oligosaccharides was by modification of a published method (21). Structural assignments were based upon previously validated methods, which included comparing retention times of unknown oligosaccharides to those of various standards, confirming the identity of sulfated structures by separate [$^{35}$S]sulfate labeling, and confirming the identity of sialylated structures by examining the change in retention times caused by neuraminidase treatment (17, 18). Recovery was consistently >90%. All samples were measured by liquid scintillation spectrometry for a time sufficient to allow for <5% counting error for major peak fractions. Serum thyroid hormone and TSH were measured by conventional RIA. Statistical analyses were performed with Student's *t* test, and significance was assigned to a two-tailed *P* value < 0.01.

## RESULTS

**RIA.** Pooled sera from perinatal animals were obtained from trunk blood of litters from pregnant rats treated with 0.05% methimazole-containing drinking water from day 16 of gestation and from litters of control rats. Although thyroid hormone ($T_4$) was not detectable in our assay in either group (<1.0 $\mu$g/dl), TSH was 9.3 ng/ml in blood from litters of methimazole-treated rats and 1.1 ng/ml in blood from litters of control animals, consistent with primary hypothyroidism in the former group. In mature methimazole-treated animals, $T_4$ was not detectable (<1.0 $\mu$g/dl), whereas in control animals, $T_4$ was 4.9 $\mu$g/dl. TSH was 21.4 ± 4 ng/ml in mature methimazole-treated animals and 2.5 ± 1 ng/ml ($P < 0.01$) in control animals—findings also consistent with primary hypothyroidism.

**Anion-Exchange HPLC.** *$\alpha$ subunit.* To characterize further the effect of congenital primary hypothyroidism on TSH carbohydrate structure, enzymatically released oligosaccharides were fractionated by anion-exchange HPLC as described. Validation of the assigned retention times for released oligosaccharides has been reported (17, 18). Representative chromatograms for perinatal TSH $\alpha$ subunit (Fig. 1 *A* and *C*) show that uncharged species, consisting of high mannose and neutral hybrid forms, were eluted in the void volume at a retention time of 3 min. Oligosaccharides with one negative charge, containing singly sialylated (N1) and sulfated (S1) species, were eluted between 8 and 12 min. Oligosaccharides with two negative charges (N2, N1S1, and S2) were eluted between 13.5 and 18 min. Oligosaccharides with three or more negative charges were eluted with a retention time > 18 min. These figures show that released oligosaccharides from perinatal euthyroid animals (Fig. 1*A*) contain anionic species with one and two charges and few forms with a retention time > 18 min; oligosaccharides from perinatal hypothyroid animals (Fig. 1*C*) contain species with one and two negative charges and a significant increase (from 2% to 7% of total; $P < 0.01$) in those species that were eluted after 18 min. These findings for TSH-$\alpha$ suggest an increase in species containing three or more negative charges in hypothyroid animals than in control animals. The means (± SEM) of quadruplicate analyses demonstrating these changes are shown in Table 1. Neutral species are not included in these analyses because they contain some residual unincorporated radioactivity in addition to high mannose forms (N.G., unpublished observations).

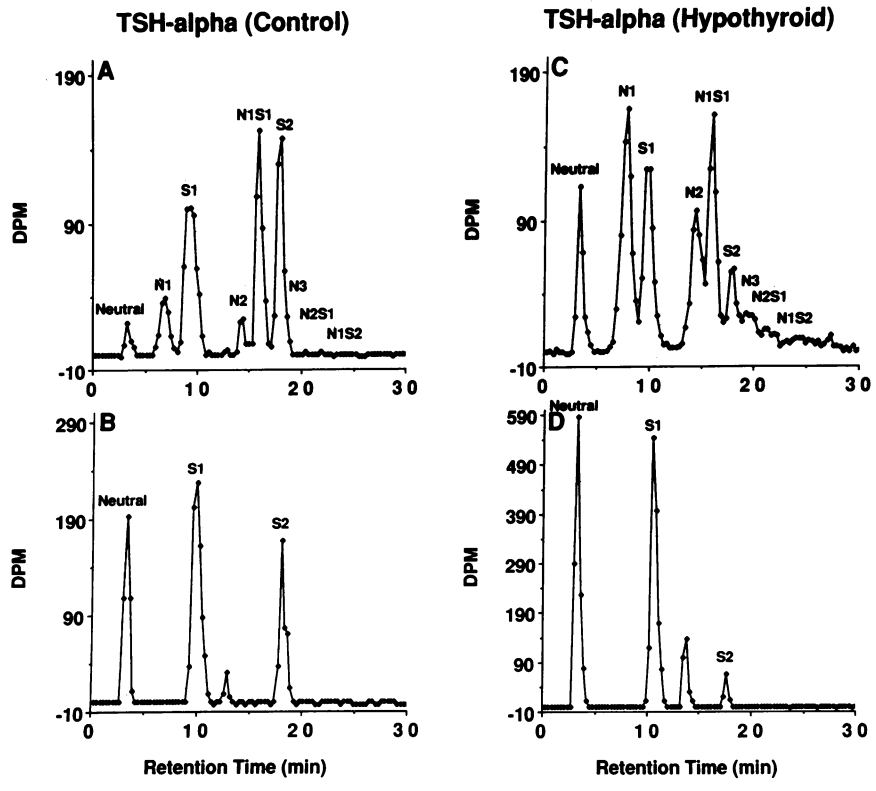


FIG. 1. Anion-exchange HPLC profiles of enzymatically released [$^3$H]GlcN-labeled secreted perinatal TSH-$\alpha$ oligosaccharides. [$^3$H]GlcN-labeled TSH-$\alpha$ oligosaccharides were chromatographed by anion-exchange HPLC from perinatal control (*A*) and hypothyroid (*C*) animals. Aliquots of oligosaccharides from perinatal control (*B*) and hypothyroid (*D*) animals were also treated with *Clostridium perfringens* neuraminidase prior to evaluation by HPLC. Expected retention times before (*A* and *C*) and after (*B* and *D*) neuraminidase are shown for sialylated (N), sulfated (S), and sialylated and sulfated (NS) oligosaccharides. Integers reflect the number of sialic acid or sulfate residues per oligosaccharide structure. A neutral species is also depicted at a retention time of 3 min. Data are normalized such that equal quantities of [$^3$H]GlcN-labeled oligosaccharides were applied to HPLC in control and neuraminidase-treated samples.

Table 1. Percent distribution of released anionic oligosaccharides (perinatal animals)

| | N1 | S1 | N2 | N1S1 | S2 | ≥3 |
|---|---|---|---|---|---|---|
| TSH-α | | | | | | |
| Control | 11 (1) | 28 (2) | 6 (1) | 27 (<1) | 26 (2) | 2 (1) |
| Hypothyroid | 24 (1) | 22 (1) | 14 (1) | 24 (1) | 9 (1) | 7 (1) |
| TSH-β | | | | | | |
| Control | <1 (<1) | 3 (1) | 1 (1) | 26 (2) | 65 (5) | 5 (2) |
| Hypothyroid | 6 (1) | 7 (1) | 10 (1) | 25 (1) | 20 (2) | 32 (2) |

Values represent the means (± SEM) of quadruplicate analyses. N, sialylated oligosaccharides; S, sulfated oligosaccharides; NS, sialylated and sulfated oligosaccharides. Integers represent the number of sialylated and/or sulfated species per oligosaccharide.

TSH-α from perinatal hypothyroid animals also demonstrated an increase in the proportion of sialylated to sulfated species compared with control animals for structures containing both one and two negative charges. Fig. 2 *Upper* shows a 2.9-fold increase in the ratio of sialylated to sulfated species with one negative charge (N1/S1) for perinatal hypothyroid animals compared with control animals ($P < 0.01$). For species containing two negative charges (N2/S2), this ratio was increased 7.4-fold for perinatal hypothyroid animals compared with control animals ($P < 0.01$). After neuraminidase digestion of perinatal TSH-α, four species were observed (Fig. 1 *B* and *D*), and sialylated forms were no longer present. A neutral species, S1, S2, and an unusual species at retention time 13 min were noted. This latter species was resistant (*i*) to 0.01 M HCl followed by alkaline phosphatase and (*ii*) to 2.0 M acetic acid, treatments respectively reported to release phosphate residues attached to mannose through mono- or diester linkages (22) and unusually linked sialic acid species (23, 24). It was also resistant to dimethyl sulfoxide in 5% methanol, a treatment demonstrated to release amine-linked sulfated oligosaccharides (25). However, treatment with 0.05 M HCl in methanol (26) resulted in the removal of this species as well as S1 and S2, suggesting that this species is sulfate-attached through a monoester linkage to an oligosaccharide. Its elution time of 13 min may reflect sulfate attached to GalNAc (or to another sugar) in other than the usual 4-O position. Finally, the proportion of sulfated species containing two negative charges (S2) was 7.7-fold greater in perinatal control animals compared with hypothyroid animals, suggesting decreased sulfation in TSH-α of perinatal hypothyroid animals.

In contrast, TSH-α oligosaccharides from mature hypothyroid animals demonstrated a small but not significant decrease (from 14% to 10% of total) compared with TSH-α in mature control animals in those species containing three or more negative charges. Moreover, TSH-α from mature hypothyroid animals demonstrated a small but not significant increase compared with control animals in the proportion of sialylated to sulfated species for structures containing both one (N1/S1 = 1.4-fold) and two (N2/S2 = 1.5-fold) negative charges. The means (± SEM) of quadruplicate analyses demonstrating these changes are shown in Table 2.

*β Subunit.* Representative anion-exchange chromatograms for perinatal TSH-β (Fig. 3 *A* and *C*) show even greater heterogeneity than for perinatal TSH-α. Congenital hypothyroidism had a dramatic effect on perinatal TSH-β glycosylation. Released oligosaccharides from perinatal TSH-β in euthyroid animals (Fig. 3*A*) contain few anionic species with one charge, a predominance of species with two negative charges, and a small percentage of forms with a retention time > 18 min; oligosaccharides from perinatal hypothyroid animals (Fig. 3*C*) contain a greater percentage of species with one negative charge and a significant increase (from 5% to 32% of total, $P < 0.01$) in those species that were eluted after 18 min. Similar to perinatal TSH-α, these findings suggest an increase in perinatal TSH-β of those species containing three or more negative charges in perinatal hypothyroid animals compared with control animals. The means (± SEM) of quadruplicate analyses demonstrating these changes are shown in Table 1.

TSH-β from perinatal hypothyroid animals also demonstrated an increase in the proportion of sialylated to sulfated species compared with control animals for structures containing both one and two negative charges. Fig. 2 *Lower* shows a 15.1-fold increase in the ratio of sialylated to sulfated species with one negative charge (N1/S1) for perinatal hypothyroid animals compared with control animals ($P < 0.01$). For species containing two negative charges (N2/S2), this ratio was increased 25.5-fold for perinatal hypothyroid animals compared with control animals ($P < 0.01$).

Following neuraminidase digestion of perinatal TSH-β, four species were observed (Fig. 3 *B* and *D*) with retention times corresponding to those observed for perinatal TSH-α. Moreover, for TSH-β the proportion of sulfated species containing two negative charges was 2.5-fold greater in perinatal control animals than in hypothyroid animals, suggesting decreased sulfation of TSH-β in perinatal hypothyroid animals.

In contrast, TSH-β oligosaccharides from mature hypothyroid animals demonstrated a significant increase (from 29% to 45% of total, $P < 0.02$) compared with TSH-β in mature control animals in those species containing three or more negative charges. Moreover, TSH-β from mature hypothyroid animals demonstrated a small but not significant decreased compared with control animals in the proportion of sialylated to sulfated species for structures containing both one (N1/S1 = 20% decrease) and two (N2/S2 = 50% decrease) negative charges. The means (± SEM) of quadruplicate analyses showing these changes are shown in Table 2.

**Specificity of Response of TSH Oligosaccharides in Congenital Primary Hypothyroidism.** Released oligosaccharides from free α subunit and total proteins were compared to those

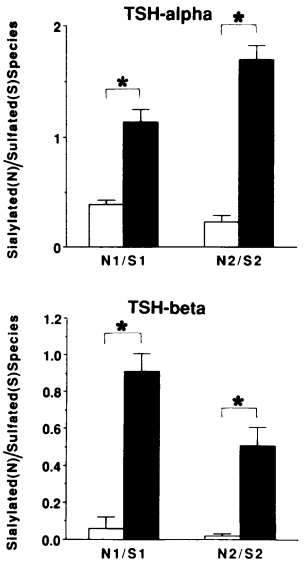


FIG. 2. Ratio of sialylated (N) to sulfated (S) perinatal TSH oligosaccharides. The ratio of sialylated to sulfated species for labeled oligosaccharides for perinatal TSH-α (*Upper*) and perinatal TSH-β (*Lower*) in control (open bars) and hypothyroid (closed bars) animals is demonstrated for species containing one negative charge (N1/S1) and two negative charges (N2/S2). Represented are the means of quadruplicate analyses. Significant differences between groups are represented by asterisks (*, $P < 0.01$).

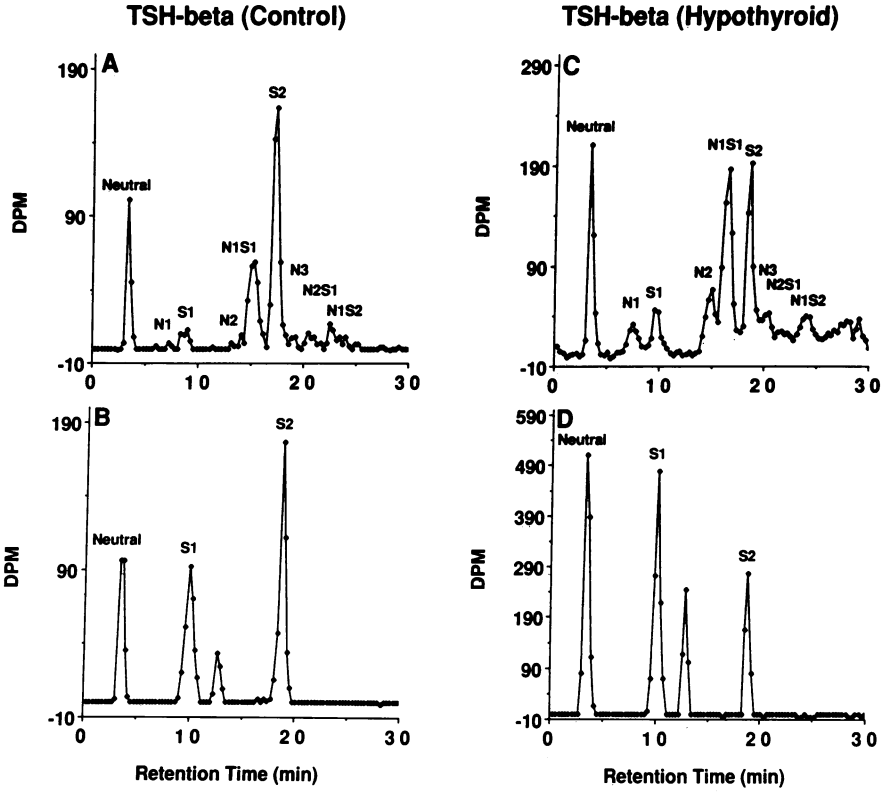


FIG. 3. Anion-exchange HPLC profiles of enzymatically released [$^3$H]GlcN-labeled secreted perinatal THS-$\beta$ oligosaccharides. [$^3$H]GlcN-labeled TSH-$\beta$ oligosaccharides were chromatographed by anion-exchange HPLC from perinatal control (*A*) and hypothyroid (*C*) animals. Aliquots of oligosaccharides from perinatal control (*B*) and hypothyroid (*D*) animals were also treated with *Clostridium perfringens* neuraminidase prior to evaluation by HPLC. Expected retention times before (*A* and *C*) and after (*B* and *D*) neuraminidase are as described in Fig. 1. Data are normalized such that equal quantities of [$^3$H]GlcN-labeled oligosaccharides were applied to HPLC in control and neuraminidase-treated samples.

from TSH subunits to evaluate the specificity of response in congenital primary hypothyroidism. In contrast to the significant increase noted for TSH subunits in the percentage of species containing three or more negative charges in hypothyroid animals compared with control animals, free $\alpha$ subunit and total proteins were unchanged (not shown). Similarly, unlike the significant increases noted for perinatal TSH subunits in sialylated species compared with sulfated species with one and two negative charges in hypothyroid animals compared with control animals (Fig. 2), these ratios in free $\alpha$ subunit and total proteins were only slightly altered. For species containing one negative charge, the ratio of sialylated to sulfated species (N1/S1) was increased 1.6- and 1.3-fold in free $\alpha$ subunit and total proteins, respectively. For species containing two negative charges (N2/S2), this ratio increased 2.0- and 1.1-fold, respectively.

## DISCUSSION

It has been established that quantitative changes in serum levels of TSH occur during ontogenesis (13–15) as well as in primary hypothyroidism (7). Moreover, we have reported previously qualitative alterations in TSH oligosaccharide structure during development (16, 17). We now report differential alterations in secreted TSH oligosaccharide structure during development in rats with congenital primary hypothyroidism. Our present studies show two findings. First, secreted TSH $\alpha$ and $\beta$ subunits from rats with congenital primary hypothyroidism, most notably in the perinatal period, have more oligosaccharides with three or more negative charges and have increased sialylation and decreased sulfation compared with control animals. Second, primary hypothyroidism results in structural changes specific to TSH, since free $\alpha$ subunit and total proteins are minimally altered in this setting.

Our data now permit a synthesis of previous indirect observations regarding the effect of end-organ removal on glycoprotein structure. We demonstrated previously that TSH secreted *in vitro* by mature hypothyroid rodent pituitary explants showed small but significant increases in multiantennary (27), as well as sialylated (28) oligosaccharide structures when compared with those from control animals. Moreover, others have reported an increase in the size and charge of intrapituitary TSH in primary hypothyroidism (29) and an increase in the size of lutropin and follitropin in castrated monkeys compared with control monkeys (30, 31). These studies have suggested indirectly that glycoprotein hormones synthesized by animals with end-organ deficiency contain oligosaccharides with increased sialylation. In the present studies, we provide direct evidence for an increase in the sialylation of both TSH-$\alpha$ and -$\beta$ oligosaccharides in rats with congenital primary hypothyroidism. The importance of sialic acid and its acceptor sugar galactose in affecting the metabolic clearance rate of glycoprotein structures has been well characterized (32–34). Consequently, the increased circula-

Table 2. Percent distribution of released anionic oligosaccharides (mature animals)

| | N1 | S1 | N2 | N1S1 | S2 | ≥3 |
|---|---|---|---|---|---|---|
| TSH-$\alpha$ | | | | | | |
| Control | 14 (1) | 18 (<1) | 11 (<1) | 34 (1) | 9 (1) | 14 (2) |
| Hypothyroid | 19 (2) | 20 (2) | 13 (1) | 30 (1) | 8 (1) | 10 (<1) |
| TSH-$\beta$ | | | | | | |
| Control | 5 (<1) | 9 (1) | 9 (1) | 27 (1) | 21 (1) | 29 (3) |
| Hypothyroid | 5 (1) | 6 (1) | 7 (<1) | 20 (1) | 17 (2) | 45 (3) |

Values represent the means (± SEM) of quadruplicate analyses. N, sialylated oligosaccharides; S, sulfated oligosaccharides; NS, sialylated and sulfated oligosaccharides. Integers represent the number of sialylated and/or sulfated species per oligosaccharide.

tory survival time reported for TSH after thyroidectomy (2) and for lutropin and follitropin after castration (30, 31) may be explained, in part, by the increased sialylation demonstrated in these studies. However, it should be pointed out that the specific mechanism by which TSH and gonadotropin clearance is prolonged by such oligosaccharide changes remains to be elucidated. Normal TSH clearance is determined predominantly by the kidney and thyroid (2) rather than the liver, which is the major site of clearance of asialoglycopeptides by the galactose receptor (32–34).

The oligosaccharide changes from our study are specific for TSH, since free $\alpha$ subunit and total proteins are minimally affected by congenital hypothyroidism. The absence of a significant change in the relative sialylation of free $\alpha$ subunit in hypothyroidism may be due to dilution by a constant pool of $\alpha$ subunit made by the gonadotrophs or to a specific effect of congenital hypothyroidism on glycosylation of TSH but not of free $\alpha$ subunit within the thyrotroph. In support of this latter possibility, we have shown previously that TSH and free $\alpha$ subunit appear to be secreted by separate intracellular pathways in the thyrotroph (35).

The present study has shown that the effect of congenital hypothyroidism on TSH oligosaccharide structure is more pronounced in perinatal animals with 1 week of fetal hypothyroidism compared with mature animals with 1 week of fetal and 8 weeks of postnatal hypothyroidism. The less dramatic structural changes we have observed in mature animals are consistent with our previous studies in both mature thyroidectomized mice (28) and rats (P.W.G., unpublished observations), where moderate structural changes were observed. It is possible that the increased sialylation that occurs with normal development (17) minimizes any further differential in sialylation in mature animals with congenital hypothyroidism.

It is also interesting that the increased sialylation of TSH oligosaccharides noted during postnatal ontogeny (17) is consistent with but less dramatic than that which we now report for perinatal hypothyroid animals. Moreover, released TSH subunit oligosaccharides from perinatal animals with congenital primary hypothyroidism contain more forms that do not bind to Con A compared with controls, suggesting the presence of more multiantennary and/or bisected structures in the former (P.W.G., unpublished observations). These data suggest the possibility that primary hypothyroidism during early development in the rat may accelerate the emergence and activity of specific sialyltransferase(s) and/or $\beta$-galactosyltransferases needed for the final processing of TSH. Such regulation would promote the synthesis of more highly branched and charged TSH oligosaccharide structures terminating in sialic acid. To what extent these events are regulated by thyrotropin-releasing hormone or other hypothalamic factors in addition to thyroid hormone deficiency is not clear. It will be interesting to investigate a molecular basis for these structural changes.

The physiological significance of a more highly sialylated TSH is not completely understood. One could speculate that increased sialylation, by delaying metabolic clearance, would augment the steady-state serum TSH concentration at any given biosynthetic rate. Moreover, since thyroid hormone is critical for neural and skeletal neonatal development, the increased biosynthesis and sialylation of TSH would provide a dual mechanism to compensate for thyroid hormone deficiency. It will be important to elucidate fully the *in vitro* and *in vivo* bioactivities as well as metabolic clearance rate and targeting of TSH from perinatal euthyroid animals versus perinatal hypothyroid animals. Such studies could clarify whether the changes in oligosaccharide structure produced by perinatal hypothyroidism constitute a rapid adaptive response to increase TSH action *in vivo* before the full effects of increased TSH subunit synthesis have been achieved.

We thank Julia Miller and Gilda Dickstein for their secretarial assistance.

1. Pierce, J. G. & Parsons, T. F. (1981) *Annu. Rev. Biochem.* **50,** 465–495.
2. Constant, R. B. & Weintraub, B. D. (1986) *Endocrinology* **119,** 2720–2727.
3. Amr, S., Menezes-Ferreira, M., Shimohigashi, Y., Chen, H. C., Nisula, B. & Weintraub, B. D. (1985) *J. Endocrinol. Invest.* **8,** 537–541.
4. Berman, S. I., Thomas, C. G., Jr., Manjunath, P., Sairam, M. R. & Nayfeh, S. N. (1985) *Biochem. Biophys. Res. Commun.* **133,** 680–687.
5. Amir, S. M., Kubota, K., Tramontano, D., Ingbar, S. H. & Keutmann, H. T. (1987) *Endocrinology* **120,** 345–352.
6. Nissim, M., Lee, K.-O., Petrick, P. A., Dahlberg, P. A. & Weintraub, B. D. (1987) *Endocrinology* **121,** 1278–1287.
7. Odell, W. D., Wilber, J. F. & Utiger, R. D. (1987) *Recent Progress in Hormone Research* (Academic, New York), pp. 47–85.
8. Shupnik, M. A., Chin, W. W., Habener, J. F. & Ridgway, E. C. (1985) *J. Biol. Chem.* **260,** 2900–2903.
9. Gurr, J. A. & Kourides, I. A. (1985) *DNA* **4,** 301–307.
10. Carr, F. E., Burnside, J. & Chin, W. W. (1989) *Mol. Endocrinol.* **3,** 709–716.
11. Wondisford, F. E., Farr, E. A., Radovick, S., Steinfelder, H. J., Moates, J. M., McClaskey, J. H. & Weintraub, B. D. (1989) *J. Biol. Chem.* **264,** 14601–14604.
12. Fisher, D. A., Dussault, J. H., Foley, T. P., Klein, A. H., LaFranchi, S., Larsen, P. R., Mitchell, M. L., Murphey, W. H. & Walfish, P. G. (1979) *J. Pediatr.* **94,** 700–705.
13. Fisher, D. A., Dussault, J. H., Sack, J. & Chopra, I. J. (1977) *Rec. Prog. Horm. Res.* **33,** 59–116.
14. Strbak, V. & Michalickova, J. (1984) *Endocrinol. Exp.* **18,** 183–195.
15. Dussault, J. H. & Labrie, F. (1975) *Endocrinology* **97,** 1321–1324.
16. Gyves, P. W., Gesundheit, N., Taylor, T., Butler, J. B. & Weintraub, B. D. (1987) *Endocrinology* **121,** 133–140.
17. Gyves, P. W., Gesundheit, N., Stannard, B. S., DeCherney, G. S. & Weintraub, B. D. (1989) *J. Biol. Chem.* **264,** 6104–6110.
18. Gesundheit, N., Gyves, P. W., DeCherney, G. S., Stannard, B. S., Winston, R. L. & Weintraub, B. D. (1989) *Endocrinology* **124,** 2967–2977.
19. Gesundheit, N., Magner, J. A., Chen, T. & Weintraub, B. D. (1986) *Endocrinology* **119,** 455–463.
20. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
21. Baenziger, J. U. & Natowicz, M. (1981) *Anal. Biochem.* **112,** 357–361.
22. Tabas, I. & Kornfeld, S. (1980) *J. Biol. Chem.* **255,** 6633–6639.
23. Green, E. D. & Baenziger, J. U. (1988) *J. Biol. Chem.* **263,** 25–35.
24. Green, E. D. & Baenziger, J. U. (1988) *J. Biol. Chem.* **263,** 36–44.
25. Nagasawa, K., Inoue, Y. & Kamata, T. (1977) *Carbohydr. Res.* **58,** 47–55.
26. Slomiany, B. L. & Slomiany, A. (1978) *J. Biol. Chem.* **253,** 3517–3520.
27. Taylor, T., Gesundheit, N., Gyves, P. W., Jacobowitz, D. M. & Weintraub, B. D. (1988) *Endocrinology* **122,** 283–290.
28. DeCherney, G. S., Gesundheit, N., Gyves, P. W., Showalter, C. R. & Weintraub, B. D. (1989) *Biochem. Biophys. Res. Commun.* **159,** 755–762.
29. Mori, M., Ohshima, K., Fukuda, H., Kobayashi, I. & Wakabayashi, K. (1984) *Acta Endocrinol.* **105,** 49–56.
30. Peckham, W. D. & Knobil, E. (1976) *Endocrinology* **98,** 1054–1060.
31. Peckham, W. D. & Knobil, E. (1976) *Endocrinology* **98,** 1061–1064.
32. Morell, A. G., Irvine, R. A., Sternlieb, I., Scheinberg, I. H. & Ashwell, G. (1968) *J. Biol. Chem.* **243,** 155–159.
33. Hickman, J., Ashwell, G., Morell, A. G., van den Hamer, C. J. A. & Scheinberg, I. H. (1970) *J. Biol. Chem.* **245,** 759–766.
34. Ashwell, G. & Morell, A. G. (1974) *Adv. Enzymol.* **41,** 99–128.
35. Stannard, B. S., Gesundheit, N., Ronin, C., Burnside, J. & Weintraub, B. D. (1988) *J. Biol. Chem.* **263,** 8309–8317.