The hypothyroid (*hyt/hyt*) mouse: A model system for studying the effects of thyroid hormone on developmental changes in gene expression

(congenital primary hypothyroidism/lipid metabolism/apolipoprotein B/gastrointestinal development)

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Communicated by David M. Kipnis, April 4, 1988

ABSTRACT Thyroid hormone has been implicated as an important factor in rodent development. We have used a strain of mice with a recessive mutation producing congenital primary hypothyroidism (C.RF/J-*hyt*/+) to study the effects of thyroid hormone on developmental changes in the expression of genes encoding a number of proteins involved in lipid metabolism and transport. Total cellular RNA was prepared from the small intestine and liver of *hyt/hyt* mice and their unaffected littermates (+/?) at various times during postnatal development. RNA blots were probed with apolipoprotein A-I, A-II, A-IV, B, and E cDNAs plus cDNAs encoding the low density lipoprotein receptor, 3-hydroxy-3-methylglutaryl coenzyme A reductase, and three cytoplasmic hydrophobic ligand-binding proteins (two fatty acid-binding proteins and a protein that binds all-*trans*-retinol). Hypothyroidism results in small changes (1.5- to 5-fold) in the concentration of many of these mRNAs in liver and small intestine between postnatal days 15 and 50. A much greater tissue-specific effect was noted on apolipoprotein B (apoB) gene expression. In euthyroid +/? animals, apoB mRNA levels fall by a factor of 30 in liver between days 20 and 35 without a comparable decrease in the small intestine. This liver-specific decrease does not occur in *hyt/hyt* animals. The normal decrease in hepatic apoB mRNA levels is accompanied by a decrease in plasma apoB-100 but not apoB-48. No reduction in either form of plasma apoB was noted in *hyt/hyt* animals. Mutant *hyt/hyt* mice given thyroxine from birth to 35 days had liver apoB mRNA levels comparable to those in +/? littermates. In contrast, hepatic apoB mRNA concentrations did not fall to normal levels in *hyt/hyt* mice given thyroxine from postnatal days 15 to 35. All treatment groups have comparable levels of plasma corticosteroids. These data suggest that (i) there is a critical period or a required response time during postnatal development for thyroid hormone action on apoB gene expression, (ii) thyroid hormone's effect on apoB is tissue specific, and (iii) the *hyt/hyt* mouse represents a useful system to evaluate the developmental effects of thyroid hormone on specific gene expression.

Since the association between cretinism and abnormal thyroid gland morphology was established (1, 2), the products of the thyroid gland have been recognized as important effectors of normal growth and development. Although postnatal brain maturation is the developmental program that is most commonly appreciated to be dependent upon the presence of thyroid hormone, it is not alone. Other organ systems, such as the rodent gastrointestinal system, have shown alterations in their developmental patterns when subjected to thyroid hormone excess or deficiency. Remarkable changes in cell

number, type, and specific gene expression have been documented in the developing intestine and liver. In rodents, many of these changes [e.g., alterations in the activities of hydrolases located in the brush border of intestinal epithelial lining cells (enterocytes) or changes in the levels of hepatic enzymes involved in gluconeogenesis and fatty acid oxidation] occur at the suckling-weaning transition concomitant with an increase in the plasma levels of thyroxine (T_4) and corticosterone. The act of weaning itself does not appear to be the inducing factor since many of these changes can occur during the same time period even when weaning is delayed (for review, see ref. 3). Studies with rats made hypothyroid with 6-propyl-2-thiouracil or by surgical removal of the thyroid gland have shown that thyroid hormone is involved in the developmental alterations in the activities of at least some of these enzymes (3, 4).

Drug or surgically induced hypothyroidism represents one commonly used approach for assessing the contribution of thyroid hormone to developmental changes in specific gene expression. Another approach, which is described in this report, employs a mutant strain of congenitally hypothyroid mice named C.RF/J-*hyt*/+ (5). The *hyt* mutation is recessive and has been mapped to a single locus on chromosome 12. Mice homozygous for this mutant gene (*hyt/hyt*) are characterized by retarded growth, infertility, mild anemia, and mildly elevated serum cholesterol (5). A number of observations have established that these animals suffer from primary hypothyroidism arising from defective development of the thyroid gland (5, 6). Histologic examination of fetal *hyt/hyt* mice has shown that this defective development is first apparent at day 18 of the 20-day gestation period (6). The thyroid gland never develops completely, remaining hypoplastic in infant and adult *hyt/hyt* animals. Serum calcium levels are normal, indicating that the parathyroid glands are functionally intact (unpublished data). Plasma T_4 concentrations are $<2 \mu\text{g/dl}$ throughout life (normal littermate T_4 levels are $6.7 \pm 1.6 \mu\text{g/dl}$) and thyroid-stimulating hormone levels are elevated up to 10-fold. Moreover, T_4 levels do not change in response to exogenous thyroid-stimulating hormone. Near normal body weight and fertility can be restored in homozygous mutants by addition of dessicated thyroid powder to the diet (5).

Abbreviations: apo, apolipoprotein; apoB, apolipoprotein B; apoB-100, apoB-48, etc., apolipoprotein B, B-100, B-48, etc., respectively; HMG CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; T_4 , thyroxine; LDL-R, low density lipoprotein receptor; L-FABP and I-FABP, liver and intestinal fatty acid-binding proteins, respectively; CRBP II, cellular retinol-binding protein II; CRIP, cysteine-rich intestinal protein.

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The power of this congenital mouse mutation is that it permits direct comparison of developmental changes in the expression of a specific gene in any tissue of affected (*hyt/hyt*) animals and their unaffected (+/?) littermates. Thus, using the hypothyroid mouse model circumvents variables inherent in surgically or chemically induced hypothyroidism and provides an ideal control group of genetically similar normal (euthyroid) littermates. To date, only one study has been published in which *hyt/hyt* animals were used to look at the developmental effects of thyroid hormone. Noguchi *et al.* (7) used *hyt/hyt* mice to define a critical period of thyroid hormone influence on cerebral development by monitoring morphologic changes in myelin basic protein and alterations in the activity of an enzyme marker of myelinogenesis (2',3'-cyclic-nucleotide 3'-phosphohydrolase).

Thyroid hormone is involved in the regulation of lipid metabolism (8). Since many of the proteins involved in lipid transport and processing are developmentally regulated in the gastrointestinal tract (9–11), we used the *hyt/hyt* mouse to examine the effects of hypothyroidism on the expression of genes encoding a number of apolipoproteins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the low density lipoprotein receptor (LDL-R), and several hydrophobic ligand binding proteins in the small intestine and liver. Our results show a dramatic delay in the normal liver-specific decline of apolipoprotein B (apoB) mRNA levels in *hyt/hyt* animals, suggesting that thyroid hormone is involved in this developmentally programmed, tissue-specific modulation of apoB gene expression.

MATERIALS AND METHODS

Animals. The C.RF/J strain of mice carrying the *hyt* gene was maintained by brother-sister matings (*hyt*/+ × *hyt*/+ or *hyt/hyt* × *hyt/hyt*—see Fig. 3 for the method used for homozygous matings). Mice were kept in a conventional mouse room with a 12-hr dark/12-hr light cycle (lights on between 0600 and 1800). They were fed pasteurized Wayne sterilizable rodent Blox (Continental Grain, Chicago) *ad libitum*.

Histology. Samples of liver, duodenum, jejunum, and ileum, prepared from 15- and 35-day-old *hyt/hyt* and +/? mice, were fixed in Bouin's fixative [40 mM picric acid/9% (vol/vol) formaldehyde/5% (vol/vol) glacial acetic acid], sectioned, and stained with hematoxylin and eosin.

RNA Blot Hybridization. Mice were sacrificed between 1000 and 1200 by decapitation. Their liver and small intestine were removed and immediately frozen in liquid N₂. Total cellular RNA was isolated from frozen pulverized tissues by using guanidine thiocyanate (12). The integrity of each RNA sample was verified by denaturing formaldehyde/agarose gel electrophoresis (13). Dot blots were prepared by applying four amounts of each tissue RNA sample (0.5–3 µg) to nitrocellulose filters as described (14). Blots were probed with ³²P-labeled (15) cloned cDNAs encoding rat apoA-I (16), human apoA-II (17), rat apoA-IV (18), rat apoB (19), mouse apoE (20), rabbit LDL-R (21), rat HMG CoA reductase (22), rat liver fatty acid-binding protein (L-FABP, ref. 23), rat intestinal FABP (I-FABP, ref. 24), rat cellular retinol-binding protein II (CRBP II, ref. 9), and a cysteine-rich intestinal protein (CRIP, ref. 25) (apoA-I, -A-II, -A-IV, -B, -E, etc. = apolipoprotein A-I, A-II, A-IV, B, E, etc.). Stringencies selected for hybridization and filter washing were identical to those detailed in ref. 10 except for human apoA-II cDNA where the hybridization and washing temperatures were reduced to 37°C and 42°C, respectively. The relative abundance of specific mRNAs in tissue RNA samples was calculated by quantitative laser densitometry of filter autoradiographs (26). Only data in the linear range of film sensitivity were used.

Immunoblot Analysis of Plasma apoB-100 and -B-48. Proteins contained in 2-µl samples of serum were fractionated by electrophoresis through 5% polyacrylamide gels containing 0.5% NaDodSO₄ (27) and then electrophoretically transferred to nitrocellulose filters at 150 mA for 15–20 hr as described (28). Blots were blocked with Blotto buffer (29, 30) and then incubated for 30 min at 23°C with a 1:1000 dilution (in Blotto) of polyclonal, monospecific rabbit anti-rat apoB serum (31). [Blotto = 5% (wt/vol) nonfat dry milk/0.01% Sigma antifoam/0.5% Tween 20/0.2% sodium azide.] Blots were washed three times (5 min per wash) with buffer A (10 mM Tris-HCl, pH 7.5/0.15 M NaCl/0.05% Tween 20) and then incubated with ¹²⁵I-labeled protein A (ICN; 36 µCi/µg; final concentration = 2 µCi/ml; 1 Ci = 37 GBq) for 30 min at 23°C. After three additional 5-min washes with buffer A, the blots were subjected to autoradiography. Bands representing immunoreactive apoB were quantitated by scanning laser densitometry (26).

Hormone Assays. T₄ and cortisol assays were performed on blood collected at the time of sacrifice. Serum was stored at –20°C. RIA kits for determining total serum cortisol and T₄ levels were from Clinical Assays (Cambridge, MA). Serum for measurement of the corticosterone levels was obtained from the retro-orbital plexus of separate litters of 15-, 20-, 35-, and 50-day-old +/? and *hyt/hyt* littermates. A corticosterone RIA kit was obtained from Radioassay Systems Laboratory (Carson, CA). Protocols from the manufacturer were used.

Serum Cholesterol. Total serum cholesterol levels were measured with a commercial assay (Wako Pure Chemical, Osaka, Japan) in serum obtained at the time of sacrifice. The manufacturer's protocol was modified slightly to use 10 µl of serum with 1 ml of color reagent solution.

Measurement of Intestinal Disaccharidase Activities. Lactase, sucrase, and maltase activities were quantitated in samples of small intestine recovered from untreated 35-day-old +/? or *hyt/hyt* mice, as described (32, 33).

Statistical Analysis. Data were assessed by a two-way analysis of variance, with phenotype and age as the variables. In those cases where this analysis indicated a significant difference ($P < 0.05$) between the *hyt/hyt* and control groups, the Student's *t* test was utilized to assess the significance of observed differences between age-matched groups of animals. A *t* value greater than $t_{0.05}$ was considered statistically significant ($P < 0.05$).

RESULTS AND DISCUSSION

Surveying *hyt/hyt* Mice and Their Normal (+/?) Littermates for Developmental Changes in Gastrointestinal Gene Expression. Livers and intestines were collected from 12-, 15-, 17-, 20-, 35-, and 50-day-old *hyt/hyt* mice and their unaffected littermates from *hyt*/+ × *hyt*/+ matings. These time points span the suckling-weaning transition (days 15–20) as well as the pubertal growth spurt (days 30–35). Histologic examination of the liver and three regions of the small intestine from *hyt/hyt* and +/? mice at 15 and 35 days revealed no gross morphologic differences between hypothyroid and euthyroid tissues. RNA was prepared from liver and intestine of up to five animals for each time point. In all cases, genotype was confirmed by measurement of serum T₄ (see Fig. 4). Dot blots of tissue RNAs prepared from individual animals were hybridized to a panel of cDNAs encoding proteins involved in lipid metabolism. They included those associated with plasma lipoproteins (apoA-I, -A-II, -A-IV, -B, and -E), HMG CoA reductase (the major regulatory protein of cholesterol biosynthesis), and the LDL-R, plus two cytoplasmic proteins abundantly represented in the gastrointestinal tract that are thought to participate in the uptake and/or metabolic processing of long-chain fatty acids (L-FABP and I-FABP; for review, see ref. 34). In addition we

also used a cDNA encoding CRIP that is induced during the suckling-weaning transition (25) plus a cDNA that encodes CRBP II that is expressed in enterocytes (9). The results of this survey are presented in Fig. 1.

Most of the differences in specific mRNA levels between normal (+/?) and homozygous (*hyt/hyt*) littermates were small (1.5- to 5-fold). Some of these differences appear to reflect developmental delays that exhibit tissue specificity. For example, in +/? euthyroid mice, apoA-I mRNA levels peak in the small intestine and liver on day 17 and begin to decline by postnatal day 20. In mutant *hyt/hyt* mice (Fig. 1), this normal decline in apoA-I mRNA is delayed ≈ 3 days in intestine and as much as 18 days in liver. A similar delay may be occurring with HMG CoA reductase mRNA in liver. CRIP mRNA increases in normal animals at the suckling-weaning transition (i.e., between days 15 and 20). This change in CRIP mRNA levels is delayed in the intestine of *hyt/hyt* mice (Fig. 1). Another pattern encountered was a more extended delay or absolute halt in the developmental progression of specific mRNA changes. This is exemplified in Fig. 1 by changes observed in liver apoE and LDL-R mRNA levels: the levels of these mRNAs fall after postnatal days 17 or 20 in the livers of +/? mice, whereas minimal reductions are seen in *hyt/hyt* animals even after postnatal day 50. Interestingly, this change

FIG. 1. Screening liver and small intestinal RNA prepared from euthyroid +/? and congenitally hypothyroid *hyt/hyt* littermates for postnatal changes in specific mRNA levels. Dot blots containing RNAs from individual animals were probed with a panel of cDNAs. The levels of specific mRNAs were determined by scanning laser densitometry of filter autoradiographs and are presented in arbitrary units. Open bars, euthyroid +/? animals; solid bars, *hyt/hyt* mice. With the exception of the 12-day time point for which a single +/? animal and a single *hyt/hyt* animal were screened, the height of the bars represent the average level of a specific mRNA in three to five animals and the error bars indicate one standard deviation. Stars above the bars indicate that the difference between mRNA levels in *hyt/hyt* and +/? littermates is statistically significant ($P < 0.05$). mRNA levels, which are indicated by a line directly overlying the x axis, were too low to be accurately quantitated. Note that the densitometric units have not been standardized from probe to probe and, therefore, the relative abundance of various mRNAs in liver or small intestine cannot be inferred from the data presented here.

in LDL-R mRNA was tissue specific: no statistically significant differences were noted in the intestines of comparably aged *hyt/hyt* and +/? mice.

Other types of changes in mRNA accumulation are also encountered in *hyt/hyt* mice. A modest increase in intestinal apoA-IV mRNA concentrations occur in the *hyt/hyt* mice at all time points surveyed after day 12. With some mRNAs (e.g., L-FABP and I-FABP), even though there are statistically significant differences in levels at some time points, it is not possible to make any generalization as to the developmental pattern. Finally, the absence of any significant change in CRBP II and apoA-II mRNA levels in intestinal RNA prepared from *hyt/hyt* and +/? animals during postnatal development suggests that alterations in the concentrations of other mRNAs do not reflect general increases or decreases in total mRNA levels in this tissue.

The Effects of Hypothyroidism on Developmental Changes in apoB Gene Expression. The most striking effect of hypothyroidism in *hyt/hyt* mice was seen with apoB. A single apoB gene codes for two serum proteins that are associated with chylomicrons (apoB-48), very low density lipoproteins, and low density lipoproteins (apoB-100). A distinctive co- or post-transcriptional nucleotide alteration results in the introduction of a stop codon into the middle of the apoB-100 gene transcript (35). This modified mRNA encodes apoB-48. ApoB-48 (210 kDa) is produced in mouse liver and small intestine whereas apoB-100 (512 kDa) is synthesized only in the liver (ref. 10 and S. Frank and A. J. Lusis, personal communication). In early postnatal life, apoB mRNA levels are similar in the small intestines and livers of *hyt/hyt* and +/? mice (Fig. 2). However, in euthyroid animals, liver apoB mRNA levels drop by a factor of 30 between days 20 and 35. This change is tissue-specific; in the small intestine there is no significant reduction. In *hyt/hyt* animals, hepatic apoB mRNA levels are elevated starting at 15 days of age and the dramatic drop in liver-specific apoB mRNA between 20 and 35 days does not occur—*hyt/hyt* liver apoB mRNA concen-

FIG. 2. Effects of hypothyroidism on developmental changes in apoB gene expression. (A) apoB mRNA levels in the small intestine and liver of euthyroid +/? mice (open bars) and *hyt/hyt* (solid bars) mice. (Inset) Representative RNA dot blot data obtained from a 35-day-old *hyt/hyt* mouse (*hyt*) and its unaffected +/? littermate (+) are included. (B) Plasma apoB-100 and -B-48 levels as determined by immunoblotting. (Inset) Pooled sera samples of three 35-day-old *hyt/hyt* mice and three 35-day-old +/? littermates.

trations remain elevated at 20, 35, and 50 days. The net result is a 30- to 50-fold higher concentration of apoB mRNA in the livers of 50-day-old *hyt/hyt* mice compared to their euthyroid +/? littermates (Fig. 2A).

Studies by Lusis *et al.* (30) have indicated that levels of apoB mRNA do not correlate with plasma apoB concentrations in various strains of mice. To examine the effects of the observed alterations in apoB mRNA levels in *hyt/hyt* mice on plasma apoB, we prepared immunoblots of samples of serum proteins fractionated by denaturing polyacrylamide gel electrophoresis. Sera were examined from 17-, 35-, and 50-day-old *hyt/hyt* and comparably aged +/? littermates (Fig. 2B). The decrease in hepatic apoB mRNA observed in +/? animals is followed by a decrease in the serum levels of apoB-100 but not apoB-48. ApoB-100 decreases by a factor of 5 from day 17 to day 35 in normal animals and is no longer detectable in the serum of 50-day-old +/? mice. In *hyt/hyt* animals, there is a decrease by only a factor of 2 in apoB-100 between day 17 and 35, and no reduction between days 35 and 50. Serum apoB-48 levels are the same in *hyt/hyt* and euthyroid +/? mice at 17 and 35 days and are reduced slightly at 50 days in *hyt/hyt* animals (Fig. 2B).

The difference between serum apoB levels in *hyt/hyt* and +/? animals does not appear to be a major contributory factor to the mild elevation in serum cholesterol observed in the mutant mice. At 17 days of age, although serum apoB levels are still identical in *hyt/hyt* and +/? animals, *hyt/hyt* serum cholesterol levels are significantly elevated (*hyt/hyt*, 242 ± 14 mg/dl; +/?, 181 ± 12 mg/dl) and do not diverge any further at 35 days as apoB-100 levels begin to decline in +/? animals (*hyt/hyt*, 176 ± 24 mg/dl; +/?, 141 ± 5 mg/dl).

"Rescuing" the Normal apoB Phenotype in *hyt/hyt* Animals.

One explanation for the apoB phenotype in *hyt/hyt* animals is that the alteration in hepatic apoB mRNA and serum protein concentration is a feature of the *hyt* mutation that is unrelated to their thyroid function. To examine this possibility, restoration of normal apoB mRNA and protein levels was attempted by supplying exogenous thyroid hormone to homozygous mutant mice. The offspring of homozygous matings were given thyroid hormone from postnatal days 15 to 35 or from birth to 35 days either orally in the form of desiccated thyroid powder or by intraperitoneal injection of T_4 (Fig. 3). When thyroid hormone was supplied from birth to 35 days by both supplementation methods, apoB mRNA levels in the livers of 35-day-old *hyt/hyt* mice were reduced to levels approximating those found in normal (+/?) animals. However, apoB mRNA levels were only reduced slightly when thyroid hormone was supplied from 15 to 35 days (Fig. 3). Corresponding changes were observed in serum apoB-100 (data not shown). These results suggest two possibilities: (i) there is a critical period before day 15 during which thyroid hormone must be present for the normal reduction in hepatic apoB mRNA concentration to occur or (ii) there is a required response time (>20 days) between exposure to T_4 and the decline in apoB mRNA levels.

Although it appears that the presence of thyroid hormone was critical for this developmental reduction in hepatic apoB mRNA levels, the possibility remains that it may not be the final hormonal effector of this alteration. Henning (36) has argued persuasively that many developmental changes that appear to be thyroid hormone dependent may in fact be corticosteroid dependent since thyroid hormone deficiency eliminates the normal increase in plasma corticosterone that occurs during the suckling-weaning transition. Early work with *hyt/hyt* mice demonstrated that the adrenocortical X-zone was poorly developed in 36- to 64-day-old mice (37). To determine whether the effect of hypothyroidism on apoB gene expression in *hyt/hyt* mice might be the result of corticosteroid deficiency, sera from +/? and *hyt/hyt* littermates were assayed for total T_4 and two corticosteroids,

FIG. 3. "Rescue" of the normal apoB phenotype in *hyt/hyt* animals treated with thyroid hormone. Rescue experiments were performed on the offspring from homozygous *hyt/hyt* matings. Mutant *hyt/hyt* mice were fed a diet supplemented with 0.025% desiccated thyroid powder to correct their infertility (6). This supplemented diet was discontinued at the first sign of pregnancy. Offspring were treated either from birth to 35 days of age or from days 15 to 35 (stippled bars). For the birth to 35-day peroral rescue experiments, a chow diet supplemented with 0.025% desiccated thyroid powder was given to mothers when the pups were born ("galactotherapy" of *hyt/hyt* animals is discussed in ref. 7). These pups were subsequently weaned to supplemented chow. For the 15- to 35-day peroral rescue experiments, the supplemented diet was not provided to either mother or pups until the postnatal day 15. An intraperitoneal injection of either saline (50 μ l) or T_4 (0.1 μ g in 50 μ g of saline) was administered daily (at 1300) to separate groups of animals either from birth to day 35 or from day 15 to day 35. The lactating mothers of this treatment group were not given a supplemented chow diet. For all treatment groups, *hyt/hyt* animals given thyroid hormone reached near normal size. Results obtained from representative litters are shown. However, in our experience, galactotherapy resulted in large variations in liver apoB mRNA levels from litter to litter. For this reason, we believe that injection is the preferred route of T_4 replacement in young animals. Open bar, euthyroid +/? mice; solid bar, untreated *hyt/hyt* mice.

cortisol and corticosterone (Fig. 4). Mutant *hyt/hyt* animals were profoundly hypothyroid at all time points examined. However, at none of the time points examined was their serum cortisol or corticosterone levels significantly different from those encountered in euthyroid +/? littermates. Moreover, in the 15-35 day and 0-35 day "rescue" experiments, the cortisol levels of *hyt/hyt* mice given T_4 were not significantly different from untreated controls (data not shown).

These data suggest that the effect of thyroid hormone replacement on apoB gene expression was not mediated by glucocorticoids. It is unclear at present why *hyt/hyt* animals do not exhibit the reduction in corticosterone levels that others have seen when they induce hypothyroidism in other rodent species and mouse strains (for review, see ref. 36). It is possible that, in the presence of a prolonged hypothyroid state, the pituitary-adrenal gland axis of *hyt/hyt* mice is able to respond and adjust corticosteroid output. The normal corticosteroid levels observed in *hyt/hyt* animals makes this model even more attractive since one can examine the effects of thyroid hormone deficiency on developmental changes in specific gene expression without the additional variable of glucocorticoid insufficiency. This fact should prove useful for evaluating the relative contributions of corticosteroids and thyroid hormone to developmental alterations in intestinal brush border disaccharidase activities. In preliminary experiments, enzymatic assay of the specific activities of lactase, maltase, and sucrase in the intestine of 35-day-old *hyt/hyt* and +/? mice indicated no significant differences between mutant animals and their euthyroid littermates (data not shown). In agreement with the work reviewed in ref. 36, these data suggest that T_4 may not be the primary hormone affecting the developmental regulation of these enzymes.

FIG. 4. Comparison of total serum T₄, cortisol, and corticosterone levels in *hyt/hyt* mice and their +/? littermates at various stages of postnatal development. T₄ and cortisol levels were measured in sera obtained at the time of sacrifice with the exception of the 35-day animals. The serum in this case was obtained from a separate litter of 35-day-old animals by collecting blood from the retro-orbital plexus. For the cortisol assay, although all littermates were bled at the same time of day, different litters were not sampled at precisely the same time of day. Therefore, given the diurnal changes that are known to occur in plasma cortisol concentrations, it is not possible to make comparisons between groups of animals at different stages of postnatal development. Blood for corticosterone assay was collected at 1300 in all cases with care being taken to minimize stress to the animals and can be compared among samples. Open bars, +/? animals; stippled bars, *hyt/hyt* animals. The cortisol assay had a 3.8% corticosterone cross-reactivity. The corticosterone assay cross-reacted 5% with cortisol.

Further studies are necessary to better characterize the mechanism(s) responsible for the timing and tissue-specificity of the apoB mRNA decline. However, a number of testable hypotheses emerge from our findings. For example, thyroid hormone is known to bind to a receptor that is located in the nuclear chromatin. This receptor is believed to be the site of initiation of thyroid hormone action (38, 39). The liver may express significant levels of thyroid hormone receptor at some critical time before the postnatal day 15. Binding of thyroid hormone to this receptor would initiate a sequence of events that results in a fall in liver apoB mRNA levels between days 20 and 35. Failure to bind thyroid hormone could result in down-regulation of the thyroid hormone receptor and/or in the failure to produce a critical intermediate in apoB down-regulation. In the intestine, the receptor may be lacking or expressed at low levels. Alternatively, in enterocytes a different receptor or proteins that modify the activity of a receptor common to enterocytes and hepatocytes may be present to "prevent" the initiation of events that result in the precipitous fall in apoB mRNA levels observed in liver. With the observation that the protein encoded by the *c-erbA* gene is likely to be a thyroid hormone receptor (40, 41), these hypotheses can begin to be explored in the *hyt/hyt* gastrointestinal tract.

We thank Drs. Aldons J. Lusis (University of California, Los Angeles) for providing recombinant plasmids encoding rat apoB and

mouse apoE, Peter Edwards (University of California, Los Angeles) for the rat HMG CoA reductase cDNA, David Russell (University of Texas, Southwestern) for the LDL-R cDNA clone, Nicholas Davidson for his kind gift of anti-rat apoB sera, and Robert Heuckeroth and Robert Duronio for their many helpful comments about the manuscript. This work was supported by Grants DK37960 and DK17947 from the National Institutes of Health. J.I.G. is an Established Investigator of the American Heart Association.

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