Starvation and Hypothyroidism Exert an Overlapping Influence on Rat Hepatic Messenger RNA Activity Profiles

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ABSTRACT To assess the effect of starvation and to explore the potential interrelationship of starvation and thyroid status at the pretranslational level, we have analyzed by two-dimensional gel electrophoresis, the hepatic translational products of starved and fed euthyroid and hypothyroid rats. 5 d of starvation resulted in a statistically significant change in 27 of 240 products visualized, whereas hypothyroidism caused a change in 20, both in comparison with the fed euthyroid state. Of considerable interest was that 68% of all changing messenger (m)RNA sequences were common to the hypothyroid and starved groups and showed the same directional shift. Further, both starvation and hypothyroidism yielded comparable decreases in total hepatic cytoplasmic RNA content. Although it has been well established that the level of circulating triiodothyronine (T_3) and the level of hepatic nuclear receptors fall in starvation, this reduction cannot account for the observed decrease of total hepatic RNA nor for all of the alterations in the concentrations of specific mRNA sequences. Thus, administration of T_3 to starved animals in a dose designed to occupy all nuclear T_3 receptors fails to prevent the fall in total RNA and the majority of starvation-induced changes in the level of mRNA sequences. Moreover, starvation of athyreotic animals results in a further decrease in total RNA and in a further change in the level of individual mRNA species. We conclude, therefore, that although the reduced levels of circulating T_3 and the nuclear T_3 receptors can contribute to the observed results of starvation, the starvation-induced changes are not exclusively mediated by this factor. The striking overlap in the genomic response between hypothyroid and starved animals raises the possibility that those biochemical mechanisms regulated at a pretranslational level by T_3 are either not helpful or injurious to the starving animal. The reduction in circulating T_3 and nuclear receptor sites together with T_3-independent mechanisms initiated in the starved animal may constitute redundant processes designed to conserve energy and substrate in the nutritionally deprived organism.

INTRODUCTION

Recent evidence suggests that nutritional status is intimately related to thyroidal status in the modulation of hepatic protein synthesis. Starvation, per se, results in a decrease of serum triiodothyronine (T_3)[1] in man and rat (1–8) and also a decrease in hepatic nuclear T_3 receptors in the rat (8–12). Associated with the effect of starvation on T_3 receptor capacity and content are alterations in the incremental response of two hepatic enzymes, malic enzyme (ME) and α-glycerol phosphate dehydrogenase (α-GPD) to a given dose of T_3 (8). Starvation and hypothyroidism are known to impair protein synthesis (13–15) and to result in a decrease in hepatic protein and RNA content (16, 17).

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[1] *Abbreviations used in this paper:* α-GPD, α-glycerol phosphate dehydrogenase; ME, malic enzyme; mRNA, messenger RNA; T_3, triiodothyronine.

Conversely, refeeding a starved animal and administration of T_3 to a hypothryoid rat effectively restore these changes to normal (16, 18, 19). The molecular basis for the impairment and restoration has not been clearly delineated. Moreover, both starvation and hypothyroidism reduce the functional messenger (m)RNA for ME (20–22), whereas starvation also reduces the functional mRNA for pyruvate kinase (23) as well as other selected proteins to a greater extent than the overall reduction in mRNA and total RNA content. Although the complexity of the mRNA sequences seems to be unchanged by either starvation (19) or hypothyroidism (24), no information is available regarding the influence of the profile of individual mRNA sequences as revealed by two-dimensional gel electrophoresis of in vitro translated products generated by poly(A)$^+$ RNA from starving rats. This technique has been successfully applied in our laboratory to study the genomic changes that occur in the rat liver as a result of manipulation of the thyroidal state of the animal (25).

The purpose of this study, therefore, was to characterize the genomic effect of starvation by identifying alterations in a specific population of in vitro translated mRNA sequences. We further wished to compare these changes with those produced by hypothyroidism. We intended to determine the degree of commonality of the influence of starvation and hypothyroidism at the genomic level and to elucidate the interrelationships between starvation and T_3 responsiveness. Since the starving rat has been extensively used as a model for the changes in iodothyronine metabolism and tissue effects that characterize patients with nonthyroidal illness, our findings may be potentially relevant to human disease.

METHODS

Poly(A)$^+$ RNA extraction and in vitro translation. Total cellular RNA and poly(A)$^+$ RNA was extracted from livers as previously described in this laboratory (20, 25, 26).

Rabbit reticulocyte lysate was prepared by the method of Evans and Lingrel (27) and treated with 40 μg/ml micrococcal nuclease (P-L Biochemicals, Inc., Milwaukee, WI) as described by Pelham and Jackson (28). In vitro translation assays were performed using the rabbit reticulocyte lysate system previously described (25). Incubations (30 μl) contained 30 μg/ml poly(A)$^+$ mRNA, 32.4 μCi[^{35}S]methionine (Amersham Corp., Arlington Heights, IL), and 22 μl lysate and were carried out for 90 min at 23°C. Incubation mixtures were centrifuged at 100,000 *g* for 1 h to pellet insoluble material. Incorporation of [^{35}S]methionine into protein was determined on a trichloroacetic acid (TCA)-insoluble aliquot of the translated products.

Two-dimensional gel electrophoresis. Aliquots (200,000 cpm) of the translated products (50–250 μg of reticulocyte lysate protein) were resolved by two-dimensional gel electrophoresis as described by O'Farrell (29) involving equilibrium isoelectric focusing in one dimension followed by sodium dodecyl sulfate (SDS) gradient electrophoresis in the second. The gels were processed for radiofluorography using En^{3}Hance (New England Nuclear, Boston, MA) as described by the manufacturers. The vacuum-dried gels were subsequently exposed to Kodak SB5 no screen medical x-ray film (Eastman Kodak Co., Rochester, NY) at −80°C and developed as described by the manufacturer. Quantitation of the translated products was accomplished by the use of videoscanning and microcomputer processing methods established by Mariash et al. (30). Since a constant number of counts was applied to each gel, the results obtained reflect the relative distribution of translational products. Since no significant difference in [^{35}S]methionine incorporation per microgram RNA in various preparations was noted, comparable translated protein was applied. Isoelectric points (pI) were determined by measurement of the pH gradient in gels focused in parallel to which no samples were applied (29). Molecular weights were determined by inclusion of ^{14}C-methylated protein standards with each SDS-gradient gel (Bethesda Research Laboratories, Rockville, MD). The translated products were initially identified in numerical sequence on the two-dimensional gels in accord with previous studies from our laboratory (25). To provide a more systematic nomenclature and in line with current practice we have subsequently identified individual sequences by the molecular size and isoelectric point ($M_r \times 10^{-3}$/pI) of the translational product.

Animal treatment. Male Sprague-Dawley rats (175–250 g) were supplied by Taconic Farms, Germantown, NY. Animals were surgically thyroidectomized by the supplier and treated by us with 100 μCi of ^{131}I (New England Nuclear) (1 μCi = 3.7×10^{10} Bq) after 1 wk of a low-iodine diet. The animals were considered to be adequately hypothyroid only after growth retardation was demonstrated 4–5 wk after ^{131}I administration. Unless otherwise stated, animals received food and water ad lib. Both euthyroid and hypothyroid animals were starved (food deprived, water ad lib) for 1 or 5 d. Animals were given a single intraperitoneal injection of T_3 (200 μg/100 g body wt) 24 h before killing at the times indicated in the text. This dose was sufficient to occupy nuclear receptors for 1 d (31). A single dose of T_3 was chosen since long-term replacement of T_3 to achieve a constant level of T_3 by infusion methods is technically exceedingly difficult. Moreover, pulsatile injections, which fail to achieve nuclear saturation, would lead to a complex analytic problem given the various half-lives of individual mRNA sequences. Since the nuclear receptor concentration in starved rats does not fall to <50% of the fed control values (8–12), and since under normal conditions nuclear sites are about one-half occupied, the calculated concentration of T_3 nuclear complex for a period of 24 h in a starved rat given 200 μg T_3/100 g body wt should equal or exceed values that characterize the fed euthyroid states.

Statistical analysis. The two-dimensional electrophoretic profiles were quantitated and then inspected visually. Differences in specific translated products among the various states were noted. Quantitation of the translated products was accomplished by determination of the mean value of the incorporated radioactivity into each spot. The number of rats per treatment (4–11) are indicated in the text. Significant differences of the various treatments was assessed by Rankit plots of the individual products for all treatments followed by multifactorial analyses of variance. Only the translated products determined to be statistically significant by paired comparisons (Student's *t* test) were noted (32). Random sampling of the products not usually identified as changing confirmed that those sequences identified as sig-

nificantly different between the physiologic states truly reflect experimentally induced significant changes ($P < 0.05$) within the populations.

To assess the significance of the similarity in responsiveness in the various states, we calculated the percentage of the total number of products that could change in the same direction simply on the basis of random alterations. Assuming that each of the 240 translated products visualized for each experimental state had an equal chance for random change, the potential random overlap for the various treatments was determined to be <2% by chi-square analyses. Thus, any overlap in directional shift >2% of the specific products between two states is significant ($P < 0.01$) (32).

Other analytical procedures. Protein was measured by the method described by Lowry et al. (33) and DNA by the method of Giles and Meyers (34). Total RNA was determined according to Fleck and Munro (35).

RESULTS

Effect of starvation and T_3 administration on hepatic RNA, DNA, and protein content. A reduction of hepatic RNA and protein content with starvation of euthyroid animals was confirmed in the present studies (Table I). A significant 34% decrease in the RNA/DNA ratio was noted with only 1 d of starvation in the euthyroid animals. This ratio fell to 50% of the base-line value after 5 d. Even though the base-line RNA/DNA ratio is diminished in hypothyroid animals compared with that in euthyroid controls, starvation caused a significant further decrease in the RNA/DNA ratio with minimum levels achieved after only 24 h. The RNA/DNA ratio in hypothyroid rats is also significantly lower after 5 d of starvation than the corresponding value in the euthyroid starved rats.

Starvation resulted in a 39% decrease in the milligrams of protein per milligram DNA in euthyroid animals after 1 d and a 33% decrease in hypothyroid animals starved for a similar period. Prolongation of fasting to 5 d resulted in a further decrease in hepatic protein of euthyroid animals, whereas further star-

TABLE I
Effect of Starvation on Hepatic RNA, DNA, and Protein Content

Group	Animal weight	Liver weight	mg protein/mg DNA	mg RNA/mg DNA	Poly(A)$^+$ RNA
	g				%
			$-T_3$		
EU-fed					
$n = 9$	234.11±43.58	9.66±1.54	71.89±11.11	2.99±0.41	2.33±0.56
EU-fast-1 d					
$n = 4$	179.75±36.20	5.43±1.17	52.70±2.21*	1.83±0.24*	2.05±0.58
EU-fast-5 d					
$n = 9$	177.44±34.35	4.45±0.29	36.74±5.16*	1.44±0.09*	2.56±0.36
TX-fed					
$n = 4$	137.75±3.86	4.08±0.76	46.92±3.28	1.24±0.05	2.30±1.01
TX-fast-1 d					
$n = 4$	131.30±2.52	3.00±0.36	35.96±1.37‡	0.83±0.05‡	2.22±1.02
TX-fast-5 d					
$n = 6$	130.80±3.76	2.53±0.13	34.93±2.13‡	0.90±0.05‡	2.37±0.44
			$+T_3$		
EU-fed	212.12±30.61	8.02±1.42	75.09±8.10§	3.01±0.23§	2.24±0.30
$n = 8$			n.s.	n.s.	
EU-fast-1 d	174.25±32.16	5.62±1.18	49.38±4.49§	1.95±0.37§	2.22±0.57
$n = 4$			n.s.	n.s.	
EU-fast-5 d	148.70±6.8	4.30±0.40	34.38±4.69§	1.41±0.08§	2.46±0.39
$n = 4$			n.s.	n.s.	

Euthyroid (EU) and hypothyroid (TX) animals were fasted for 0, 1 or 5 d. Hepatic RNA (mg RNA/mg DNA) and protein content (mg protein/mg DNA) were determined ($-T_3$). To separate groups of animals, a single dose of T_3 (200 μg/100 g body wt) was administered to EU animals ($+T_3$) at the onset of 0 d (EU-fed) or 1 d of starvation (EU-fast-1 d) or on day 4 of a 5-d starvation (EU-fast-5 d). All data is expressed as mean±SD.

* $P < 0.05$ compared with euthyroid fed control (EU-fed).

‡ $P < 0.05$ compared with hypothyroid fed control (TX-fed).

§ Comparison made with untreated euthyroid groups ($-T_3$).

vation in the hypothyroid animals did not result in an additional loss of protein. Moreover, the level of milligrams protein per milligram DNA was similar in euthyroid and hypothyroid animals deprived of food for 5 d. These findings are similar to those previously reported from this laboratory and are consistent with a diminished pool of labile hepatic protein in hypothyroid animals, which is rapidly depleted by 1 d of starvation (16).

To assess further the potential contribution of altered serum T_3 and nuclear receptor concentrations in the diminished RNA and protein content due to starvation, a single receptor-saturating dose of T_3 (200 μg/100 g body wt) was administered intraperitoneally to euthyroid animals at the onset of a 1-d starvation or after 4 d of a 5-d starvation. Hepatic RNA, DNA, and protein content were measured 24 h later. The administration of T_3 at the onset of a 1-d starvation period had no influence on the starvation-induced decrease in either RNA or protein content. Moreover, T_3 administered on the 4th d of starvation did not alter the RNA and protein content when measured on the 5th d of starvation (Table I).

None of these treatments significantly altered the total DNA content. Moreover, the percent poly(A)$^+$ RNA, as estimated by oligo (dT) cellulose chromatography, was comparable for all treatments (2.05–2.56%). Thus, changes in RNA and protein content in starvation appear not to be primarily due to altered T_3 levels.

Two-dimensional gel electrophoresis of translated products. Representative two-dimensional electrophoretic patterns are illustrated in Fig. 1. These patterns reflect relative translational activity of various poly(A)$^+$ RNA sequences; equal counts per minute (200,000) were applied to each gel. Many products appear to respond similarly in starvation and hypothyroidism compared with the euthyroid fed control. For example, sequence 17.5/4.9 (spot 14) clearly present in the euthyroid fed state, was diminished or absent in the euthyroid fasted and hypothyroid fed states. The visual detection of these similarities stimulated us to provide a quantitative assessment of the pretranslational effects of starvation and hypothyroidism.

The effects of starvation and hypothyroidism on specific products are listed in Table II. Both increased and decreased relative amounts of specific mRNA are observed compared with the euthyroid fed state. The magnitude of the shift from base line varies for each product and is not necessarily the same in the transitions from the euthyroid fed to the hypothyroid and

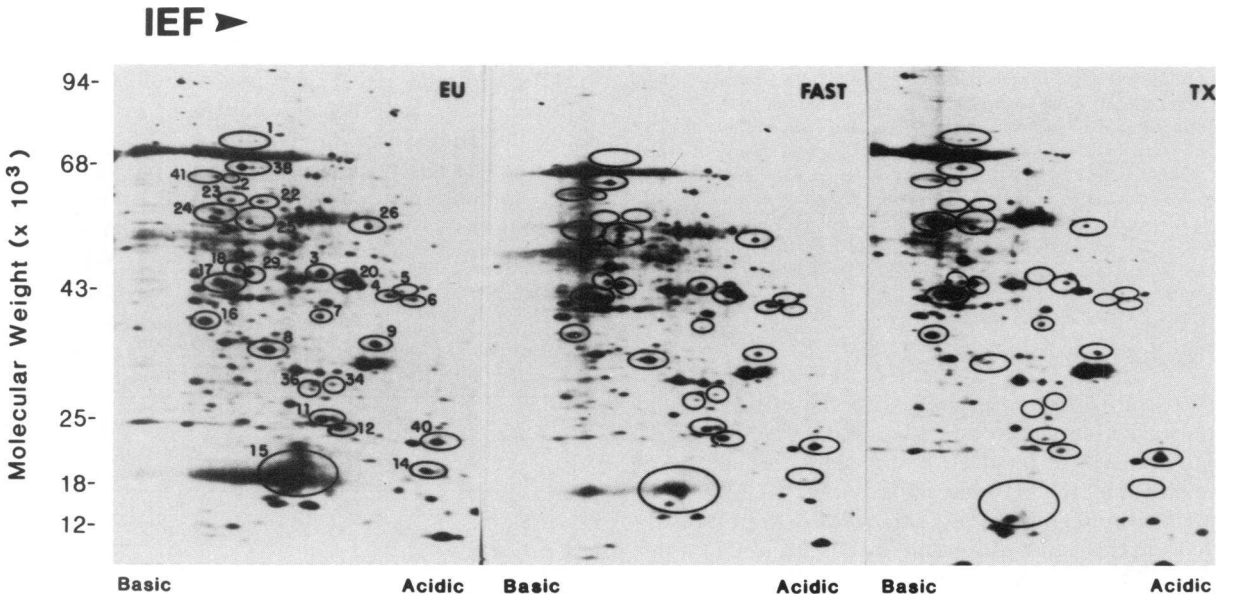

FIGURE 1 Two-dimensional gel electrophoresis of translated products. Representative fluorograms of two-dimensional gel electrophoresis of [^{35}S]methionine-labeled translated products of hepatic poly(A)$^+$ RNA obtained from euthyroid rats fed ad lib. (EU), euthyroid rats fasted for 5 d (FAST) and hypothyroid rats fed ad lib (TX) are shown. The isoelectric focusing range was from pH 7.9 (*left*) to pH 4.5 (*right*); sodium dodecyl sulfate migration was from top to bottom, with range indicated. The 28 translated products circled represent consistent alterations from the euthyroid fed state. Equal counts per minute (200,000) were applied to each gel; all gels were processed identically. Products identified with numbers <20 are consistent with those previously identified as T_3-responsive by Seelig et al. (25).

TABLE II
Effect of Starvation and Hypothyroidism on Specific mRNA Sequences

$M_r \times 10^{-3}$	pI		EU-fed*	TX-fed	EU-fast (5 d)
72	7.1	1	36.0±11.7	70.6±4.18*	92.6±20.5*
64.5	7.2	38	81.0±27.5	133.1±33.5	224.8±31.9*
62.5	7.4	41	164.5±46.8	119.7±11.7	250.8±28.7*
62	7.4	2	73.7±16.2	13.7±3.31*	45.8±4.7*
59	7.2	23	38.4±12.5	22.4±14.2	14.6±12.4*
59	6.9	22	34.3±13.7	2.3±2.7*	12.7±9.5*
55–57	7.4	24	124.2±52.2	362.6±132.9	351.0±86.8*
55–57	7.0	25	78.9±35.2	137.4±50.1	241.1±90.0*
56	5.7	26	94.4±32.4	108.6±45.3	47.8±15.1*
44	7.1	18	84.2±17.4	29.0±11.7*	53.8±10.4*
43	7.0	29	222.0±54.5	337.6±54.1*	347.4±28.5*
43	6.2	3	243.4±32.3	2.43±3.55*	171.0±20.5*
41.5	5.9	20	339.0±88.3	90.0±35.7*	340.0±96.3
40	7.4	17	547.0±68.8	962.1±168.7*	1096±138.7*
41	5.3	5	46.4±10.5	14.1±17.7*	18.7±9.5*
40	5.5	4	170.9±42.5	17.0±21.9*	60.7±13.8*
38	5.2	6	48.5±16.2	12.9±4.7*	11.1±8.4*
36	6.2	7	67.4±8.2	31.0±6.3*	11.6±1.9*
33.5	7.5	16	85.7±23.0	192.5±32.9*	174.6±27.8*
31.5	5.8	9	182.1±23.4	43.8±27.1*	62.1±33.3*
30	6.7	8	217.6±17.8	15.0±20.7*	172.6±17.8*
26	6.2	36	30.0±7.2	17.2±9.9	8.0±7.0*
25	6.4	34	27.5±5.2	22.3±16.9	14.5±5.8*
22.5	6.1	11	185±24.5	64.0±28.4*	123.7±10.0*
22.0	6.0	12	154.5±21.5	27.2±30.5*	111.6±15.9*
20.5	4.8	40	167.9±28.7	422.1±72.7*	262.1±64.0*
17.5	4.9	14	118.4±48.3	23.6±16.7*	4.0±5.2*
16	6.2–7.4	15	2254.6±312.1	94.3±118.6*	728.3±53.30*

Effect of starvation and hypothyroidism on specific mRNA sequences. Relative translational activity expressed as counts per minute per gel of the specific hepatic mRNA sequences obtained from fed euthyroid animals (EU-fed, n = 11), fed hypothyroid animals (TX-fed, n = 7), and 5-d fasted euthyroid animals (EU-fast-5 d, n = 7) are summarized. Values were obtained by quantitation of individual two-dimensional electrophoretic patterns with equal counts per minute applied to each gel. Molecular weights ($M_r \times 10^{-3}$) were determined from ^{14}C-labeled standard proteins run in parallel and pI as described by O'Farrell (29). Data are expressed as mean±SD.

* $P < 0.05$, comparison with EU-fed.

starved states. In comparison with the fed euthyroid state, starvation resulted in a significant change in 27 and hypothyroidism in 20 of the 240 products visualized (Table III). Of particular note, 19 of the 20 sequences influenced by hypothyroidism were also affected in the same direction by starvation (14 decreased, 5 increased), representing a 68% concordance in the directional shift of the combined total of the 28 changing products. No products changed in the opposite direction thus yielding 0% discordance (Table IV). Further, 1 of 28 sequences was altered by hypothyroidism but not by starvation and 8 of 28 sequences by starvation but not by hypothyroidism. 32% of the changes were therefore designated as "unrelated."

Examination of the potential role of T_3 in mediating the starvation-induced changes in translational products. Although the administration of T_3 resulted in no significant change in total RNA content, the significant pretranslational overlap noted between starvation and hypothyroidism suggested to us the possibility that starvation could have resulted in the alterations of specific mRNA sequences exclusively by reduction in circulating T_3 and T_3 receptors. We have examined this possibility with the same experimental design discussed above in connection with our studies to assess the role of T_3 in mediating the starvation-associated diminution in total RNA content. In essence, we used four experimental tests: (A) to determine whether a dose of T_3 administered at the beginning of starvation and designed to saturate the nuclear sites for 1 d could prevent or reverse starvation-induced changes of individual sequences occurring on that day; (B) to determine whether a similar saturating dose of T_3 administered on the 4th d of starvation could cause a significant reversal of the starvation-induced changes; and (C and D) to determine whether starvation of athyreotic animals could produce a further shift in any mRNA sequence beyond that induced by hypothyroidism alone.

The results of the individual tests were classified as "compatible" or "incompatible" with exclusive T_3 mediation of the starvation-induced changes by strictly defined criteria (Appendix). All results were subjected to statistical analysis. If a response could not be formally classified as either compatible or incompatible, it was designated as "indeterminate." For an overall evaluation of the responses as compatible with exclusive T_3 mediation, not one of the constituent tests could designate the response as incompatible. The results of these analyses are summarized in Table V. When each

TABLE III
Comparison of Euthyroid-to-Hypothyroid Transition with Euthyroid Fed-to-Euthyroid Fasted Transition

Transition	Spots		Total spots altered	Total spots visualized
	Decreased	Increased		
EU → TX	15	5	20	240
Fed → Fast	18	9	27	240

Within the population of translated hepatic poly(A)$^+$ sequences as depicted by two-dimensional radiofluorography, comparisons were made between euthyroid fed (n = 11) and hypothyroid fed (n = 7) animals (EU → TX) and between euthyroid fed (n = 11) and euthyroid fasted 5 d (n = 7) animals (Fed → Fast). Mean values of multiple determinations of the incorporated radioactivity into each spot were compared for each of the treatments. The translated products with altered counts per minute values statistically significant ($P < 0.05$) by paired comparisons are summarized.

TABLE IV

Pretranslational Overlap of Euthyroid-to-Hypothyroid Transition (A) and Euthyroid Fed-to-Fasted (5 d) Transition (B)

Transition	A B ↓ ↓	A B ↑ ↑	A B ↓ ↑	A B ↑ ↓	A B ↓ 0	A B ↑ 0	A B 0 ↓	A B 0 ↑	Total products
(A) EU → TX (B) Fed-Fast	14	5	0	0	1	0	4	4	28
	Concordant 67.89%		Discordant 0%		Unrelated 32.14%				

Translated products significantly ($P < 0.05$) increased (↑), decreased (↓), or unaltered (0) by hypothyroidism (A) and starvation (B) as described in Table II are summarized. The percent concordance reflects the percentage of the total changing products altered in the same direction for both transitions.

of the four tests was applied to each of the changing mRNA sequences, the alterations of only one sequence (44/7.1) were consistent with exclusive T_3 mediation. All remaining sequences were incompatible by at least one of the three test described.

The conclusion that the changes in only one sequence during starvation is compatible exclusively with a diminished T_3 effect should not be construed as implying that a decrease in serum T_3 and nuclear receptors could not play an important contributory role in the alterations in the activity of certain mRNA sequences. Thus, we found three sequences (36/6.2; 22/6.0; 17.5/5.0) to be more sensitive to T_3 during starvation than in the fed state. In each instance, the administration of 200 μg T_3 on the first day of starvation maintained the euthyroid fed levels and on the fourth day returned the level of the sequence into the euthyroid range. Nevertheless, the level of these sequences in hypothyroid animals was even further reduced by starvation, thus implying the operation of a T_3-independent mechanism.

TABLE V

Potential Mediation by T_3 of Starvation-induced Changes in Euthyroid Animals

Criteria	No. of sequences			
	Compatible	Incompatible	Indeterminate	Total changes
EU-fast-1 d	0	5	7	12
EU-fast-5 d	10	4	13	27
TX-fast-1 d	18	9	—	27
TX-fast-5 d	15	12	—	27
All criteria	1	6	20	27

Translated products that are significantly altered with starvation of euthyroid animals at 1 (EU-fast-1 d) or 5 d (EU-fast-5 d), are categorized as compatible or incompatible with exclusive T_3 mediation of starvation-induced changes according to the criteria A and B, respectively, set forth in Appendix Table I. If a response could not be classified formally as compatible or incompatible, it was designated as indeterminate. The 27 products significantly altered with starvation of euthyroid animals for 5 d were then categorized as to compatibility with exclusive T_3 regulation of the changes by the effect of starvation in hypothyroid animals for 1 and 5 d (criteria C and D, Appendix Table I).

DISCUSSION

The studies described clearly demonstrate a striking similarity, at a pretranslational level, between the genomic response of rat liver to the stimulus of hypothyroidism and to the stimulus presented by a 5-d period of starvation. Of the 240 translational products visualized, 5-d starvation caused a statistically significant change in 27, and hypothyroidism a change in 20 from the base-line euthyroid fed state. 68% of all changing sequences were common for both states and changed in a similar direction. These observations can thus be added to the previously established list of similarities in response pattern elicited by both stimuli including a decrease in total RNA, poly(A)$^+$ RNA, and total cellular protein (8–18, 23).

In assessing the significance of the observed changes in the activity of the mRNA sequences, several points should be considered. The 240 sequences demonstrated are presumably those most abundantly expressed in the rat liver (36) and consequently the changes observed may not reflect the genomic responses as a whole. Further, the alteration in mRNA levels determined by an in vitro translation system may not correspond to translational activity in vivo nor to the protein levels observed in the intact animal. Moreover, translational processing, which is known to be influenced by starvation (37–39), may be a regulatory factor in protein synthesis. In addition, no inference can be made from our studies as to whether the observed effects on translational activity of individual mRNA sequences are due to alterations in the rates of transcription, processing of mRNA precursors, or turnover

of the individual sequences. Despite the limitations in these analysis, the experimental approach allows us to compare a larger fraction of the in vivo translatable hepatic genome than has heretofore been possible. The patterns generated point to a striking similarity between hypothyroidism and starvation, which cannot be explained simply by random variation in the intensity of the translational activity of individual mRNA sequences.

We addressed ourselves to the question of the potential contribution of the combined effects of a reduction of circulating T_3 and nuclear T_3 receptor sites to the changes in total RNA, poly(A)$^+$ RNA, and specific mRNA sequences induced by starvation. A large receptor-saturating dose of T_3 administered at the onset of the first day of starvation does not influence the decrease in protein and RNA during the first day. Administration of a similar dose of T_3 on the fourth day of starvation also is without effect. Moreover, starvation of hypothyroid animals causes a further fall in the level of total RNA. We therefore conclude that the fall in total RNA and protein in starvation is not due primarily to a reduction of circulating T_3 and nuclear T_3 receptors.

It appeared possible, however, that a lowering of T_3 and nuclear receptors could have mediated the starvation-induced alterations of individual mRNA sequences. Our data, however, also make it unlikely that the observed alterations are due exclusively to a diminished T_3 effect. With only one product (44/7.1) were the changes observed entirely compatible with such exclusive T_3 mediation. Nevertheless, a number of sequences appear to be very sensitive to T_3 in the starved state. Thus, although the fall in T_3 and receptors could play a prominent role in the alterations of the activity of certain mRNA sequences, the changes observed in the mRNA activity profile are not mediated entirely by a diminished T_3 effect.

Since hypothyroid animals are known to exhibit a reduced food intake when compared with the euthyroid state (40), the possibility arose that alterations observed in hypothyroidism could be attributed simply to diminished food consumption. Nevertheless, this appears unlikely, since the level of total RNA and many specific mRNA species are lower in thyroidectomized-starved rats than in euthyroid animals subjected to starvation alone. Moreover, the fractional reduction of total RNA produced by 1 d of starvation was nearly the same in hypothyroid and euthyroid animals. Again, these findings do not rule out the possibility that the diminished food intake makes a partial contribution to the RNA changes in hypothyroidism.

The biological significance of the overlapping genomic response by hypothyroidism and starvation remains speculative. Both hypothyroidism and starvation are characterized by a diminished activity of RNA polymerase I and II (19, 23) and reduced protein and RNA synthesis (13–17). These alterations may be related either as cause or effect to the changes in the mRNA activity profiles observed in our study.

The genomic alterations in starvation clearly may also have an adaptive value inasmuch as they could result in a reduction of those biochemical processes that are either unimportant or harmful to the nutrient-deprived animal and in a stimulation of these processes that are important to survival. For example, synthesis of fat during a period of nutrient deprivation would be clearly counterproductive. Since T_3 is known to stimulate the formation of lipogenic enzymes, a reduction in circulating T_3 and T_3 receptor would serve a useful purpose. As we have previously indicated, starvation may also be responsible for inhibition of lipogenic enzyme formation by mechanisms other than reduction of T_3, such as an increase in glucagon and a reduction in insulin (22, 41, 42). Thus, it appears entirely possible that certain genes are regulated both by T_3 and by T_3-independent pathways in starvation. Such redundancy would appear to be useful as a "fail-safe" mechanism in protecting the nutrient-deprived animal. In this connection it is interesting to note that in starvation there is a reduction in oxygen consumption that is clearly not dependent on a decrease in T_3 production, since a comparable reduction is observed in starved hypothyroid animals maintained on a constant daily dose of T_3 (43). Similar response patterns have been observed in hepatic nuclear proteins (44).

It is apparent that starvation and hypothyroidism are distinctive clinical entities. Although our studies reveal a strong similarity in mRNA profiles, these profiles were far from identical. Moreover, it is entirely possible that many of the individual mRNA sequences present, which are too low in supply to be detectable by our methods, may actually have changed in an opposite direction in hypothyroidism and starvation. If so, this would provide a further explanation of the physiological difference between the two states as well as an explanation for the increased sensitivity of the mitochondrial enzyme α-GPD to the administration of T_3 during starvation (8). The complexity of the alterations is even further increased when it is recognized that three sequences hyperrespond to T_3 during starvation as compared with the fed normal state.

The starving animal has frequently been used as an implicit model of the changes in iodothyronine metabolism and action that occur in patients with nonthyroidal disease. It would be of considerable interest, therefore, to test the adequacy of this assumption by analyzing the mRNA profile in animal models of nonthyroidal disease such as the tumor-bearing rat (45) and the uremic rat (46). Such studies could conceivably

APPENDIX TABLE I

Criteria for Exclusive T_3 Mediation of Decreases in mRNA Sequences Induced by Starvation

	Tests	Criteria		
		Compatible	Incompatible	Indeterminate
T_3 to fasting euthyroid animals	A. Day 1	$(ES_1)' \geqq EF$ and $(ES_1)' \neq ES_1$	$(ES_1)' < EF$ and $(ES_1)' \leq ES_1$	$(ES_1)' \geq EF$ and $(ES_1)' = ES_1$
	B. Day 5	$(ES_5)' > ES_5$	$(ES_5)' < ES_5$	$(ES_5)' = ES_5$
Starvation of hypothyroid animals	C. Day 1	$ES \geqq HF$ and $HF = HS_1$	$HF > ES_1$ or $HF > HS_1$	— —
	D. Day 5	$ES_5 \geqq HF$ and $HF = HS_5$	$HF > ES_5$ or $HF > HS_5$	— —

EF, euthyroid fed ES_1, ES_5, euthyroid starved 1 and 5 d, respectively; HF, hypothyroid fed; HS_1, HS_5, hypothyroid starved for 1 and 5 d, respectively; $(ES_1)'$, $(ES_5)'$, euthyroid animals starved 1 and 5 d, respectively, but treated with maximal dose of T_3 1 d before killing. Responses that fit neither compatible nor incompatible were designated as indeterminate. When the shift caused by starvation of euthyroid animals was positive rather than negative, change all > to < and < to > signs.

also succeed in identifying genomic changes common to these models and the model under consideration in this report.

APPENDIX

Four tests were used to determine whether starvation-induced changes in the activity of specific mRNA sequences could have been mediated exclusively by the starvation-associated reduction in the level of circulating T_3 and the content of hepatic nuclear T_3 receptor sites: (A) T_3 was administered in a dose sufficient to saturate the nuclear receptors for the first day of starvation (200 μg); (B) a similar dose of T_3 was injected at the beginning of the fifth day of starvation; and (C and D) thyroidectomized ^{131}I-treated animals were starved for 1 or 5 d, respectively. Strict criteria were drawn up for each test to determine whether a given mRNA activity response is compatible or incompatible with exclusive T_3 mediation (Appendix Table I). If a given response could not be designated as either compatible or incompatible, it was classified as indeterminate. For an overall evaluation of a response as compatible with exclusive T_3 mediation, not one of the tests could designate the response as incompatible. The classification scheme for mRNA products falling in response to starvation are summarized in Appendix Table I. The significance of changes between groups was evaluated by the Student's *t* test.

As is indicated in Appendix Table I, when nuclear sites are fully saturated by injected T_3 for the first day, the necessary but not sufficient (compatible) criteria require that the value at the end of the first day of starvation in euthyroid animals treated with T_3, $(ES_1)'$, should equal or exceed the value in the euthyroid fed animal EF. In other words, the maximal dose of T_3 should prevent the starvation-induced fall. At the same time $(ES_1)'$ has to be significantly greater than the value achieved after 1 d of starvation without concomitant treatment ES_1, i.e., it cannot be statistically indistinguishable from ES_1. If because of scatter of the data $(ES_1)' \geq EF$ and $(ES_1) < EF$ but $(ES_1)'$ is not statistically greater than ES_1, the result is designated as "indeterminate." The criteria for "incompatibility" are $(ES_1)' < EF$ and $(ES_1)' \leq ES_1$.

Different criteria are applied to test B when nuclear sites were saturated for the fifth day of starvation. In essence, the criteria listed in Appendix Table I place any significant increase in the mRNA activity over untreated, starved animals in the "compatible" category. On the other hand, if T_3 administration causes no change, it is considered "indeterminate", since no information is available about the temporal response characteristics of sequence under study. The criteria used with respect to tests C and D, starvation of athyreotic animals, are self-explanatory and simply depend on the conclusion that if the animal is completely deprived of T_3, starvation should produce no further changes as long as all starvation-induced changes are mediated by a diminished T_3 effect. If starvation instead of causing a diminution of a given mRNA activity level causes an increase, the criteria in Appendix Table I should be reversed as indicated in the footnote.

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