Studies with Mouse Pituitary Thyrotropic Tumors

IX. Distribution of Amino Acids under the Influence of Thyroxine[1]

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SUMMARY

The effects of thyroxine on the percentage distribution *in vivo* of C^{14} lysine in mouse pituitary thyrotropic tumors have been studied. Tumor-bearing mice received 15 μc of L-lysine-U-C^{14} by injection. The distribution of isotope was determined in 5 cellular fractions of tumor and in several normal tissues. The percentage distribution of labeled amino acid into histone fractions of 2 strains of responsive tumors was depressed when tumors were grown in the presence of thyroxine. There was no effect of thyroxine on the percentage distribution of histone in a nonresponsive tumor strain, when compared to the distribution in untreated control tumors. The distribution of isotope in liver, kidney, and muscle from tumor-bearing mice did not appear to be affected by the presence or absence of thyroxine. The histones of tumors and of normal tissues were analyzed for amino acid composition. No significant differences were noted among the several tumor strains and the normal tissues studied. The relationship of these results to the mechanism of thyroxine effect on these tumors is discussed.

Gorbman (9) first noted the appearance of pituitary tumors following radiothyroidectomy in mice. Later, Furth *et al.* (8) discovered that such tumors were capable of secreting thyrotropin and isolated from them both dependent and autonomous strains, responsive and nonresponsive to thyroxine. Detailed studies have been made in this laboratory (10, 11, 14–16) with 3 strains of thyrotropic tumor obtained from Dr. Furth. Growth of 1 strain, dependent responsive, 4183, is suppressed by the administration of thyroid hormone to the host. Tumor cell implants of this strain can be suppressed by thyroxine treatment and will remain viable for long periods of time, as shown by the appearance of a tumor when treatment is discontinued (15). In another strain, autonomous responsive, L24, growth is arrested by thyroxine treatment when the tumor is small, i.e., 1 cm or less in size, but not when it is larger. Growth of the 3d strain, autonomous nonresponsive, L23, is not inhibited by thyroxine; in fact, this tumor appears to grow at a more rapid rate when thyroxine is available.

The present experiments were designed to determine whether these differing effects of thyroxine upon growth might be reflected by differences in protein formation within the several tumors as well as in the amino acid composition of these proteins. The lysine C^{14} content of histones decreased in the dependent responsive tumor when growth was suppressed by thyroxine; no similar changes occurred in the other tumors, nor was there any alteration in amino acid incorporation.

MATERIALS AND METHODS

Tumors were grown in hypothyroid female LAF_1 mice by techniques described elsewhere (15). Some of the mice received 0.12% thyroxine in the diet for 12 days prior to the study. L24 tumors were used when still small enough to be suppressible by thyroxine treatment. Thyroxine treated and untreated animals, bearing the same tumor strain, were studied at the same time. Each experiment was repeated twice again, at later dates due to the long time required to complete the determinations on a single tumor strain (Table 3).

Tumor-bearing mice (20–30 gm) received 15 μc L-lysine-U-C^{14} (Schwarz BioResearch, Inc., specific activity 130 mc/mole) I.P. At the desired time interval, the animals were anesthetized and killed by exsanguination. Tumors, and in some cases, liver, spleen, striated muscle, and kidney, were immediately removed, rinsed in ice-cold saline and weighed. The tissues were handled individually; in

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some experiments, the same tissues from several animals were pooled after weighing. All subsequent procedures were performed at 4°C.

The tissues were homogenized in 0.25 M sucrose with a glass tube and pestle (1). After filtration through cheesecloth, the homogenate was fractionated by differential centrifugation. A washed nuclear pellet was obtained by centrifugation at 700 × *g* for 10 min., followed by resuspension and resedimentation, twice. The combined supernatants were then centrifuged at 8500 × *g* for 30 min. The sediment from this centrifugation, representing the mitochondrial fraction, was resuspended once. The resulting supernatant fluids were pooled and centrifuged at 105,000 × *g* for 1 hr. The microsomal pellet formed from this centrifugation was rinsed with sucrose, but it was not resedimented. Material remaining in the final supernatant was dialyzed against distilled water for 8 hr. to separate free amino acids from soluble proteins.

Histones were extracted from the washed nuclear fraction in cold 0.25 N HCl (approximately 5 ml/gm of original tissue weight) for 30 min. with occasional stirring. The extract was then centrifuged at 37,000 × *g* for 30 min. The resulting supernatant fluids contained the histones. Previous experiments showed that the dialyzable material in the histone fraction was negligible; the normal dialysis step was therefore omitted in order to improve quantitation (R. Mandl, unpublished results; and reference 7). The over-all procedure is standard throughout the literature.

Histones, mitochondrial, microsomal, and soluble protein fractions were hydrolyzed in boiling 6 N HCl for 15 hr. Aliquots of each fraction were then analyzed for C^{14} content in a Nuclear Chicago D-47 β-counter and for amino acid content by a Technicon amino acid analyzer (Technicon Chromatography Corporation, Chauncey, New York).

RESULTS

Intracellular Distribution of Isotope

Percentage distribution of L-lysine-U-C^{14} in 5 cellular fractions of tumors are listed in Table 1. Since the distribution did not seem to vary with time, an average for each group has been made. It is apparent that thyroxine treatment of the host mouse reduced the percentages of labeled lysine in the histones of the responsive, 4183, strain of tumor from that in 4183 tumors growing in untreated hypothyroid control mice. A similar response was noted in the L-24 strain although there were no control animals run concurrently for comparison. Decrease was accompanied by an increase in the percentage of radioactivity present in the supernatant fractions.

Thyroxine treatment had no effect upon the autonomous nonresponsive L23 tumor. Here, the relative amount of isotope in the tumor histones was, if anything, slightly greater than that in L23 tumors from untreated hypothyroid animals. Also, no difference in isotope distribution was observed in the other cellular fractions of the various tumors whether from treated or untreated hypothyroid hosts.

In a few experiments, the isotope distributions among 5 cellular fractions of liver, kidney, and striated muscle from the tumor-bearing mice were analysed (Table 2).

TABLE 1

Percentage Distribution of Isotope in Cellular Fractions of Thyrotropin-producing Pituitary Tumors

Tumor strain	Treatment	No. of mice	Hr. after injection	Distribution in cell fractions (%)				
				Histone	Mitochondrial	Microsomes	Soluble protein[a]	Dialysate[a]
Nonresponsive (L23)	T4	1	1½	23	10	23	38	6
		3	2	16	17	26	38	4
		2	2	15	14	27	40	4
		3	2	23	15	25	34	4
		1	24	26	8	27	36	3
Average				20	13	26	37	4
Nonresponsive (L23)	None	1	1	14	15	23	45	4
		3	2	19	12	29	30	10
		3	2	20	16	27	34	3
Average				18	14	26	36	6
Responsive (4183)	T4	3	2	9	17	27	43	4
		3	2	11	16	20	44	9
		2	2½	11	10	33	46	2
Average				10	14	27	44	5
Responsive (4183)	None	3	2	16	19	27	35	4
		2	2	24	18	26	31	1
		2	3	16	11	29	41	3
Average				18	16	27	36	3
Responsive (L24)	T4	1	2	9	8	31	48	4
		1	24	10	6	33	50	2
Average				10	7	32	49	3

[a] From dialysis of the 105,000 × *g* supernatant.

The presence or absence of thyroxine had no consistent effect on the percentage distribution of lysine in histones of these tissues, and the type of tumor carried by these animals had no effect on percentage distribution in the histone fraction of these normal tissues.

Compared to normal tissues, the percentage of isotope in tumor histones was not abnormally high. In fact, the lowered percentage in the histone fraction of the responsive tumors, following thyroxine feeding, resulted in lower values than for any other tissue studied. However, the percentage distribution of isotope in the nonresponsive tumor histones, regardless of whether the host mice had received thyroxine in the diet, was comparable to that in liver and kidney histones, although somewhat lower than that in muscle histones.

Specific Activity of Cellular Fractions

Certain trends may be noted in the specific activities of the proteins in the 4 cellular fractions of the autonomous nonresponsive and the dependent responsive tumors (Table 3). The data in Table 3 are presented in paired form for the histone fraction but have been pooled for the other fractions. Considerable variation in specific activities was observed from 1 experiment to another with both treated and untreated tumor pairs of either responsive or autonomous tumor strains. Dependent responsive tumors from thyroxine-treated mice had a lower specific activity in the histone fraction than that in either the untreated dependent responsive tumor controls, or any of the other tumors. This corresponds to the reduction in percentage of isotope present in this fraction. The specific activity of the microsomal fraction from thyroxine-treated dependent responsive tumors was suggestively decreased although this was not clearly demonstrated by the pooled data. No alteration occurred in the mitochondrial or soluble protein fractions.

Amino Acid Composition of Tumor and Tissue Protein

Amino acid analyses were made of the histones from each of the 3 thyrotropic tumor strains. In a few instances, liver, kidney, spleen, and muscle histones were also analyzed. The amino acid composition was found to be essentially the same in the 3 tumor and 4 normal tissues studied (Table 4). In agreement with other workers (3–

TABLE 2

Percentage Distribution of Isotope in Cellular Fractions of Liver, Kidney, and Muscle from Tumor-bearing Mice

Tissue	Type of tumor	Treatment	Hr. after injection	Distribution in cell fractions (%)				
				Histone	Mitochondria	Microsomes	Soluble protein	Dialysate
Liver	Nonresponsive (L23)	T4	1½	20	22	26	31	2
		T4	24	22	12	27	37	2
		None	1	23	20	22	32	2
	Responsive (L24)	T4	1½	17	32	19	26	6
		T4	24	22	27	20	30	2
Kidney	Nonresponsive	T4	24	19	13	22	42	4
		None	1	18	20	18	34	12
Muscle	Responsive (L24)	T4	24	43	5	5	37	11
		None	1	50	9	4	19	18

TABLE 3

Specific Activities of Proteins after Injection of Radioactive L-Lysine

Specific activities of proteins in counts/min./mg protein dry weight in 2 strains of tumor, 2–3 hr. after injection of radioactive L-lysine. Paired experiments were conducted with tumors from thyroxine treated and untreated hosts. Values for each of 3 experiments are given, with ranges in parentheses, except for the histone fraction for which the values are given for individual pairs.

Strain	Treatment	Histone experiment			Mitochondria	Microsomes	Soluble protein
		I	II	III			
Nonresponsive (L23)	T4	623	1034	780	1056 (665–1566)[a]	2139 (531–2948)	1524 (891–2660)
	None	580	1085	670	1633 (1487–1779)	2400 (1238–3891)	1394 (1095–1897)
Responsive (4183)	T4	406[b]	545[b]	952[b]	1326 (1156–1518)	1831 (1189–2285)	1512 (836–2233)
	None	615	760	1143	1081 (1009–1180)	2028 (1116–3063)	1421 (979–2233)

[a] $P = 0.05$ when the paired experiments are tested for significance.

[b] $P < 0.01$ for mean decrease from corresponding untreated tumors.

TABLE 4

AMINO ACID COMPOSITION OF TUMOR, SPLEEN, AND KIDNEY HISTONES

Values are expressed as percentage of total amino acids recovered. No corrections have been made for hydrolytic losses. Spleen and kidney histones were obtained from animals bearing the nonresponsive tumor.

Tissue	Nonresponsive (L23)	Nonresponsive (L23)	Responsive (4183)	Responsive (4183)	Responsive (L24)	Kidney	Spleen
Treatment	None	T4	None	T4	T4	None	None
No. of determinations	2	3	1	1	2	1	1
Amino Acids							
Aspartic	9.7	9.7	8.5	9.5	9.0	8.7	8.9
Threonine	5.2	5.4	5.4	5.6	6.1	5.4	5.3
Serine	6.1	6.0	6.2	6.9	7.1	6.2	6.3
Glutamic	8.9	10.0	9.9	10.3	9.9	8.3	10.7
Proline	5.5	5.5	5.7	4.5	4.6	6.1	6.3
Glycine	8.7	8.5	8.4	9.0	9.9	7.9	8.0
Alanine	10.7	10.6	10.8	11.6	11.7	12.7	12.0
Cysteine	0.5	—	0.6	—	—	—	—
Valine	6.7	6.4	6.4	5.9	6.7	6.3	6.9
Methionine	1.4	1.5	1.1	0.6	0.8	1.0	0.9
Isoleucine	3.2	3.5	3.4	3.1	3.3	3.8	4.1
Leucine	9.2	9.0	9.2	9.0	8.6	8.9	8.2
Tyrosine	2.1	2.1	2.2	2.0	1.9	1.8	1.8
Phenylalanine	3.7	3.3	3.4	3.0	2.9	2.9	2.6
Lysine	9.2	9.3	9.7	10.3	9.8	10.9	9.3
Histidine	4.6	3.7	3.9	3.9	3.5	3.4	2.8
Arginine	4.8	5.7	5.2	5.0	5.1	5.7	5.7
Lysine / Arginine	1.9	1.6	1.9	2.1	1.9	1.9	1.6
Basic / Acidic	1.0	0.9	1.0	1.0	1.0	1.2	0.9

6), the histones contained large quantities of alanine, dicarboxylic amino acids, and basic amino acids, especially arginine and lysine. The ratio of basic to acidic amino acids in the present data has been close to 1 in all tissues studied. In most instances, there was about twice as much lysine as arginine in the histones.

DISCUSSION

Less lysine-C^{14} was present in the histone fractions of a dependent responsive mouse pituitary tumor strain, and probably of an autonomous responsive strain as well, when the thyroidectomized host mice were fed thyroxine than was present when the host mice were untreated. Tumor growth of these responsive strains is known to be inhibited by thyroxine feeding (8). In contrast, no change occurred in an autonomous nonresponsive tumor strain following thyroxine administration.

Abnormalities in histone metabolism of tumor cells have been described previously. Rotherham *et al.* (13), working with a dye-induced hepatoma in rats, found the specific activity of histones in the tumor to be somewhat higher than that in normal liver, whereas the specific activities of other nuclear proteins in these tumors were lower.

Busch *et al.* (1), working with Walker and Jensen tumors in rats, reported that a considerably higher percentage of radioactive lysine was incorporated within the tumor histones than in the histones of any normal tissue studied except muscle. Specific activities of the tumor histones also were found to be quite high in comparison to the histones of normal tissue and exceeded those of any cytoplasmic fraction, a relationship unlike that in the other tissues studied.

The present results differ somewhat from those of Busch *et al.* Despite the fact the thyrotropic tumors studied grew well in untreated hypothyroid hosts, the percentage distribution of lysine-C^{14} in the histone fraction was within the same range as that in the normal tissues analyzed. Moreover, there was an actual reduction in specific activity of the histones of the responsive tumors upon treatment of the hosts with thyroxine.

The decrease of lysine-C^{14} in the histone fraction could possibly be related to inhibition of thyrotropin secretion by the tumor cells upon treatment of the host with thyroxine. Such suppression occurs as well in responsive mouse pituitary thyrotropic tumors as it does in the normal pituitary (10).

It is more likely, however, that the inhibitory influence of thyroxine on the lysine-C^{14} content of the histone fraction may be related to its inhibitory effect on tumor growth. Thus, thyroxine suppresses thyrotropin secretion in nonresponsive tumors, yet it does not inhibit tumor growth (10); also, the lysine-C^{14} content of the histone fractions of these tumors was not suppressed.

One other study is closely related. Moolten and Scott (12) have used labeled precursors of purines and pyrimidines to study the effect of thyroxine on nucleic acid synthesis in both responsive and nonresponsive strains of mouse thyrotropic tumor. They found that the synthesis of both RNA and DNA was suppressed by thyroxine treatment of hosts bearing responsive tumors but not nonresponsive tumors. No change in the specific activity of the total tumor protein was demonstrated.

In the present study, the amino acid distribution in the tumor histone fraction was the same for both responsive and nonresponsive tumor strains. This does not exclude the possibility that differences may have existed. The histone fraction consists of many different protein species, and a subtle change in any one of these might have occurred and not been made evident in an analysis of the whole fraction. Qualitative differences among histones of neoplastic cells have been described by others. Davis and Busch (6) found that Walker tumors incorporated radioactive lysine into a histone fraction not present in normal tissues. Despite this, amino acid analysis of the unfractionated histones failed to reveal any abnormality (2).

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