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Research Article

Benign and malignant nodules in human thyroid glands, which did not concentrate iodide in vivo, were also unable to accumulate iodide in vitro. The mean thyroid-to-medium ratio (T/M) in seven benign nodules was 0.8±0.2 compared with 7±2 in adjacent normal thyroid tissue. In four malignant thyroid nodules, the mean T/M was 0.5±0.1 compared with 11±4 in adjacent normal thyroid. Despite the inability of such nodules to concentrate iodide, iodide organification was present but was only one-half to one-third as active as in surrounding normal thyroid. Thyroid-stimulating hormone (TSH) increased iodide organification equally in both benign nodules and normal thyroid although it had no effect in three of the four malignant lesions. The reduction in organification is probably related to the absence of iodide transport, since incubation of normal thyroid slices with perchlorate caused similar diminution in iodide incorporation but no change in the response to TSH. Monoiodotyrosine (MIT) and di-iodotyrosine (DIT) accounted for most of the organic iodide in both the nodules and normal tissue. The MIT/DIT ratio was similar in normal and nodule tissue. The normal tissue contained much more inorganic iodide than the nodules, consistent with the absence of the iodide trap in the latter tissue. The thyroxine content of normal thyroid was 149±17 μg/g wet wt and 18±4 μg/g wet wt in the nodules. The transport defect […]

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ABSTRACT Benign and malignant nodules in human thyroid glands, which did not concentrate iodide in vivo, were also unable to accumulate iodide in vitro. The mean thyroid-to-medium ratio (T/M) in seven benign nodules was 0.8±0.2 compared with 7±2 in adjacent normal thyroid tissue. In four malignant thyroid nodules, the mean T/M was 0.5±0.1 compared with 11±4 in adjacent normal thyroid. Despite the inability of such nodules to concentrate iodide, iodide organification was present but was only one-half to one-third as active as in surrounding normal thyroid. Thyroid-stimulating hormone (TSH) increased iodide organification equally in both benign nodules and normal thyroid although it had no effect in three of the four malignant lesions. The reduction in organification is probably related to the absence of iodide transport, since incubation of normal thyroid slices with perchlorate caused similar diminution in iodide incorporation but no change in the response to TSH. Monoiodotyrosine (MIT) and diiodotyrosine (DIT) accounted for most of the organic iodide in both the nodules and normal tissue. The MIT/DIT ratio was similar in normal and nodule tissue. The normal tissue contained much more inorganic iodide than the nodules, consistent with the absence of the iodide trap in the latter tissue. The thyroxine content of normal thyroid was 149±17 μg/g wet wt and 18 ±4 μg/g wet wt in the nodules. The transport defect in the nodules was not associated with any reduction in total, Na^+-K^+- or Mg^{++}-activated ATPase activities or the concentration of ATP. Basal adenylate cyclase was higher in nodules than normal tissue. Although there was no difference between benign and malignant nodules, the response of adenylate cyclase to TSH was greater in the benign lesions.

These studies demonstrate that nonfunctioning thyroid nodules, both benign and malignant, have a specific defect in iodide transport that accounts for their failure to accumulate radioactive iodide in vivo. In benign nodules, iodide organification was increased by TSH while no such effect was found in three of four malignant lesions, suggesting additional biochemical defects in thyroid carcinomas.

INTRODUCTION

We previously reported that the failure of thyrotropin (TSH) [1] to increase in vivo ^{131}I uptake in benign nonfunctioning or "cold" thyroid nodules cannot be ascribed to defective binding of TSH or activation of the adenylate cyclase-cyclic AMP system (1). Basal- and TSH-responsive adenylate cyclase activities of such nodules were significantly greater than in the adjacent normal thyroid tissue. Although stimulation of iodide uptake and organification by TSH appears to be mediated by the adenylate cyclase-cyclic AMP system (2–4), the lack of iodide uptake and response to TSH in benign "cold" nodules does not reflect a generalized abnormality in the action of cyclic AMP. TSH increased colloid droplet formation and [1-^{14}C]glucose oxidation in benign "cold" nodules as in adjacent normal thyroid (1). Although controversy exists as to whether augmented ^{32}P

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[1] *Abbreviations used in this paper:* DIT, diiodotyrosine; MIT, monoiodotyrosine; T_4, thyroxine; T/M ratio, thyroid-to-medium ratio; TSH, thyroid-stimulating hormone.

incorporation into phospholipids induced by TSH is dependent on cyclic AMP (5), this parameter was also more responsive to TSH in "cold" nodules.

Since this initial study compared effects of TSH on ^{131}I uptake in "cold" nodules in vivo and other metabolic responses to TSH in vitro, subsequent studies were directed towards correlating the latter response with iodine metabolism in vitro. Preliminary experiments demonstrated defective iodide transport in "cold" nodules in comparison with adjacent normal thyroid tissue though organification of iodide was only moderately decreased in this tissue compared with the normal (6). DeGroot has also reported that "cold" nodules were unable to concentrate iodide in vitro, but could incorporate ^{131}I into protein (7).

METHODS

The criteria for selection and study of patients and the preparation and incubation of tissue have been reported (1). Iodide transport and organification were examined by the method of Ahn and Rosenberg (3). For iodide transport, thyroid slices were incubated initially for 1 h in Krebs-Ringer phosphate buffer (pH 7.4) in an atmosphere of air at 37°C in a Dubnoff metabolic shaker. Slices were then incubated for 30 min in 4 ml of the same buffer containing 1 mg/ml glucose, 0.5 mg/ml albumin, 1 μg/ml I^- as KI, 0.25 mg/ml Tapazole, and 0.05 μCi/ml ^{131}I. At the end of the incubation, the tissue slice was rinsed in cold saline, blotted, and digested in 1 ml of 2 N NaOH, and an aliquot of the digest and of the medium was counted. In preliminary experiments with normal thyroid tissue, the thyroid to medium ratio (T/M) obtained at 30 min was similar to that found at 120 min. However, since time curves were not done with nodular tissue or with each normal thyroid studied, the T/M ratios do not necessarily represent equilibrium values. The effect of perchlorate on iodide transport in normal thyroid tissue was examined by inclusion of 1×10^{-2} M sodium perchlorate in the buffer during a 20 min incubation preceding the 30 min incubation mentioned above. Sodium perchlorate was also present during the latter incubation. During the 20 min incubation, the buffer also contained 1 mg/ml glucose and 0.5 mg/ml albumin. Organification of iodide was studied as follows. Thyroid slices were incubated for 1 h in Krebs-Ringer phosphate buffer and then incubated for 30 min in 2 ml of the same buffer containing 1 mg/ml glucose, 0.5 mg/ml albumin, 1 μg/ml I^- as KI, and 2.5 μCi/ml ^{131}I. At the end of the incubation, the slices were homogenized in 3 ml of cold 10% trichloroacetic acid (TCA). The precipitate was extracted twice with 5 ml of 10% cold TCA and then dissolved in 1 ml of 2 N NaOH and counted.

The tissue uptake and distribution of iodine was also measured using the methods of Ahn and Rosenberg (3) except that the tissue was incubated for 90 min with approximately 15 μCi of ^{131}I. The labeled components of the thyroid were identified using methods previously described (8). Briefly, the tissue slices were rinsed quickly in saline and then homogenized in cold saline-Tris buffer (0.003 M Tris, 0.11 M sodium NaCl), pH 8.5, containing 0.05 M methylmercaptoimidazole as described by Inoue and Taurog (9). Digestion was carried out with 1.75 mg Pronase for 18 h under anerobic conditions at 37°C as described by these authors. Labeled [^{125}I]thyroxine (T_4) was added at the start of the digestion to monitor recovery. After digestion, 0.14 ml of methanol/concentrated NH_4OH (1:1 vol/vol) was added, the tubes were centrifuged, and the supernate was decanted. Residual ^{131}I and ^{125}I in the precipitate were less than 10% of the total and were virtually completely extracted by a second wash of methanol/ammonia. 50–100 μl of this supernate was then chromatographed on Whatman 3MM paper (3M Co., Photographic Products Div., St. Paul, Minn.) in two systems; tertiary amyl alcohol (TAA)/hexane/2 normal NH_4OH (5:1:6, vol/vol/vol) and butanol(BAA)/2 N acetic acid (1:1, vol/vol). After drying, the strips were cut into 2-cm sections and counted in an automatic gamma-well counter with suitable correction for the overlap of ^{131}I counts in the ^{125}I spectrometer window (about 10%). Identification of [^{131}I]T_4 was made by location of [^{125}I]T_4 in the TAA system. The [^{131}I]monoiodotyrosine (MIT) and diiodotyrosine (DIT) were identified by reference to the migration of known unlabeled compounds chromatographed periodically in the same system (BAA) and identified by staining with diazotized sulfanilic acid. Origin material on BAA chromatographs was less than 1% in experiments using both normal slices and tissues from nodules. Results were expressed as nanograms I incorporated per milligram DNA using the concentration of iodide in the initial incubation medium (6×10^{-6} M) to calculate the specific activity. The T_4 content of the pronase hydrolysates was estimated by immunoassay as previously described (10).

The effect of perchlorate on iodide transport and organification in normal thyroid tissue was examined by inclusion of 1×10^{-2} M sodium perchlorate in the incubation medium. The ATP concentration of tissue was determined in triplicate by the method of Ohta, Jarrett, and Field (11). Total ATPase activity and the ouabain-sensitive Na^+-K^+-, and Mg^{++}-activated components were assayed in whole thyroid homogenates by the procedure of Stanbury, Wicken, and Lafferty (12). Adenylate cyclase activity was measured in triplicate in homogenates, as previously described, in the presence and absence of 1 mU bovine TSH/ml (13). Cyclic AMP was measured as previously described (1). DNA was measured by the method of Burton (14). The limited amount of tissue did not always permit measurement of every variable in each nodule and adjacent normal thyroid tissue. The results are expressed on the basis of DNA since this is probably a better indication of the cell content of the tissue than either protein or wet weight. TSH (2 U/mg) was kindly provided by the National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.

RESULTS

The data in Table I summarize the results for iodide transport and organification in vitro in 11 patients with nonfunctioning thyroid nodules. Seven patients had benign lesions, and four had papillary carcinoma with some follicular elements. Slices of normal thyroid tissue from each patient concentrated iodide. In contrast, slices from nonfunctioning nodules, both benign and malignant, did not concentrate iodide. Although one of the nodules had a T/M ratio of 2, this was considerably less than in the adjacent normal thyroid tissue. Extracellular space measurements were not made in both tissues but expansion of this space cannot explain the differences in results between the control

TABLE I

Comparison of Iodide Transport and Organification in "Cold" Thyroid Nodules and Adjacent Normal Thyroid

Patient	Diagnosis	Iodide transport T/M		Iodide organification			
				Normal		Nodule	
		Normal	Nodule	Basal	TSH	Basal	TSH
				ng I/mg DNA			
Su	Follicular adenoma	6	0.8	26±1	38±2	9±0.3	20±1
Ha	Nodule in multinodular gland	10	0.7	60±5	87±3	81±9	130±8
Ju	Follicular adenoma	8	0.5	41±1	64±1	59±3	87±1
Ch	Embryonal adenoma	7	2.0	51±3	64±5	8±0.4	14±1
Mo	Follicular adenoma	15	0.5	166±12	454±3	55±3	126±5
An	Follicular adenoma	20	0.6	41±17	119±24	13±1	39±6
Om	Hurthle cell adenoma	2	0.7	15±2	35±4	10±1	12±1
Mean ±SEM		7±2	0.8±0.2	57±19	123±56	34±12	61±20
Wi	Papillary carcinoma	21	0.4	243±8	320±19	27±2	31±4
He	Papillary carcinoma	5	0.5	45±4	78±4	37±8	37±3
Le	Papillary carcinoma	12	0.7	267±9	484±70	131±42	147±23
Maf	Papillary carcinoma	4	0.3	57±14	146±41	7±0.6	12±2
Mean ±SEM		11±4	0.5±0.1	153±59	257±91	51±28	57±31

The TSH concentration was 50 mU/ml. The values for iodide organification are the mean ±SEM of triplicate determinations.

tissues and the slices from the nodules. It is possible that differences in extracellular space could account for the minor differences between the T/M ratios in the various nodules.

Organification of iodide by slices of normal thyroid varied considerably. In all of the patients, 50 mU/ml TSH significantly augmented organification of iodide in normal thyroid tissue, and there was no apparent

TABLE II

Distribution of Iodine Compounds Formed during In Vitro Incubation of Tissue from "Cold" Thyroid Nodules and Adjacent Normal Thyroid

Patient	Diagnosis	Condition	Normal				Cold nodules			
			I^-	MIT	DIT	MIT/DIT	I^-	MIT	DIT	MIT/DIT
			ng I/mg DNA							
Ha	Adenoma	Control	1,220	48	28	1.7	156	30	6	5.0
		TSH	891	101	47	2.1	151	48	10	4.8
Mo	Adenoma	Control	2,090	42	14	3.0	12	30	8	3.8
		TSH	1,530	69	23	3.0	18	95	42	2.3
Sp	Adenoma	Control	670	107	32	3.3	146	37	11	3.4
		TSH	607	204	69	3.0	138	38	10	3.8
An	Adenoma	Control	723	36	16	2.3	81	15	6	2.5
		TSH	678	101	39	2.6	92	33	15	2.2
Le	Carcinoma	Control	994	49	20	2.5	276	16	5	3.2
		TSH	638	66	29	2.8	56	8	3	2.7
Mean		Control	1,139	56	22	2.6	134	26	7	3.6
SEM			257	10	3	0.3	44	4	1	0.4
Mean		TSH	869	108	41	2.6	91	44	16	3.2
SEM			173	25	8	0.2	25	14	7	0.5
TSH/Control, %			80	193	186		68	169	228	

The values are the means of triplicate determinations. The TSH concentration was 50 mU/ml.

TABLE III

T_4 Content of Tissue from "Cold" Thyroid Nodules and Surrounding Normal Thyroid Tissue

Patient	Normal	Nodule
	μg T_4/g wet wt (mean ±SEM)	
Ha	114±8	23±4
Sp	195±42	5±1
An	134±32	24±3
Le	153±9	20±9
Mean ±SEM	149±17	18±4

Four to six specimens were used for each determination.

difference between patients with benign or malignant lesions. Cells of the nonfunctioning nodules, both benign and malignant, organified iodide even in the absence of an iodide-concentrating mechanism. TSH significantly stimulated organification of iodide in six of the seven benign lesions, and the percent increment induced by TSH was similar in normal and benign nodular tissue. In contrast to these results, TSH had no effect on organification of iodide in slices from three of the four carcinomas and only a small effect in the fourth carcinoma. To determine whether or not there were qualitative as well as quantitative differences in iodine organification between the nodules and normal tissues during the in vitro incubation, pronase digestion and chromatography of the [^{131}I]labeled compounds were carried out separately in five patients (four with follicular adenomas and one with papillary carcinoma). Virtually all labeled iodine in these tissues was found in the form of iodide and MIT and DIT, though small quantities of labeled T_4 were sometimes found in extracts of normal slices (Table II). Most of the radioactivity was in the form of iodide in both types of tissue. The mean iodide concentration in the five normal tissues represented 93.6% of the total ^{131}I whereas in the five nodules it was 80.2% of tissue ^{131}I. The marked difference in iodide content is further evidence of the defect in iodide transport characteristic of the "cold" thyroid nodules. During incubation with TSH, there was a small but statistically significant ($P < 0.05$) decrease in the content of iodide in the normal slices but no significant effect on iodide content in the tumor tissue. The mean quantities of MIT and DIT formed in the normal tissues were two- to threefold greater than in the nodules. The MIT/DIT ratio in the normals was not significantly different from that in the nodules under control conditions. Incubation with TSH resulted in a 86 and 93% increase in the incorporation of iodine into MIT and DIT in the normal tissue and a 68 and 128% increase in MIT and DIT formation in the nodular tissue. However, one adenoma (Sp) and the one carcinoma (Le) did not respond to TSH during in vitro stimulation. As in the studies using TCA precipitation to measure iodide organification (Table I), less iodide was organically bound in the tissue from the "cold" nodules. However the response to TSH was similar in the nodular and paranodular tissue in the two groups.

Since iodothyronine formation was difficult to detect in vitro, the T_4 content of both the normal and nodular tissue was examined to determine whether iodothyronine synthesis was also decreased in vivo. The mean T_4 content of the four samples of normal tissue was approximately eight times that of the "cold" nodules (Table III). This would suggest that despite the only moderate decrease in iodide organification demonstrated in vitro in these "cold" nodules, there was considerably less T_4 formed in vivo.

To determine the effects of inhibition of iodide transport on organification in tissues from normal thyroid, comparative studies were done in the presence and absence of sodium perchlorate, a competitive inhibitor of iodide transport. Incubation with perchlorate markedly reduced the T/M ratio in comparison with control slices, although it did not reach a level of 1.0 as would have been anticipated (Table IV). The explanation for this is unknown but it is unlikely that there is organically bound iodine since even in the absence of methimazole, over 90% of the label is in the form of inorganic iodide. This reduction of iodide transport was associated with a decrease in basal iodide organification to 45% of the control level, but the response to TSH was unaltered. Thus, these results are qualitatively similar to the alterations in iodine metabolism that were observed in the nodules.

TABLE IV

Effect of Sodium Perchlorate on Iodide Transport and Organification in Normal Thyroid Tissue

Patient	Perchlorate	Iodide transport, T/M	Iodide organification	
			Basal	TSH
			ng I/mg DNA	
Un	−	8.0	47±3	96±1
	+	2.0	25±3	42±1
Ap	−	4.0	58±6	115±6
	+	1.7	27±3	33±1
Le	−	12.0	267±9	484±70
	+	0.3	111±4	220±34
Fe	−	18.0	38±3	103±5
	+	3.5	14±5	41±3
Mean ±SEM	Perchlorate, %	22±8	45±3	40±4
	Control			

The iodide transport and organification were determined in triplicate. The concentration of perchlorate was 10 mM and TSH was 50 mU/ml.

TABLE V

ATPase and ATP Concentration in "Cold" Thyroid Adenomas and Adjacent Normal Thyroid

Patient	Diagnosis	ATPase activity						ATP concentration	
		Total		Ouabain-sensitive Na^+-K^+-activated		Mg^{++}-activated			
		Normal	Tumor	Normal	Tumor	Normal	Tumor	Normal	Tumor
		μmol P_i liberated/mg DNA/60 min						*nmol/mg DNA*	
Ju	Follicular adenoma	2.9	7.9	0.6	1.6	1.0	2.0	—	—
Tu	Follicular adenoma	1.7	5.1	0	0.1	0.7	2.0	52±4	131±18
Ch	Embryonal adenoma	2.8	3.0	0.3	0.1	0.7	1.5	90±19	229±21
Mo	Follicular adenoma	5.1	13.7	1.3	1.3	1.0	3.0	364±17	390±15
Un	Follicular adenoma	3.5	5.2	1.0	1.3	1.2	0.6	111±15	58±3
Mean ±SEM		3.2±0.6	7.0±1.9	0.6±0.2	0.9±0.3	0.9±0.1	1.8±0.4	154±71	204±72

The ATPase assays were done in duplicate and those for ATP in triplicate.

Although the biochemical steps involved in iodide trapping have not been elucidated, the process is energy-dependent (15). A role for a ouabain-sensitive Na^+-K^+-activated ATPase in this process has also been suggested (16), though a recent study did not find a stoichiometric relationship between these two processes (17). No consistent difference between ouabain-sensitive Na^+-K^+-activated ATPase activity was found in the nonfunctioning and normal thyroid tissue (Table V). The total and Mg^{++}-activated ATPase was actually significantly higher in the tissue from the nodules compared with normal. Furthermore, ATP concentrations were similar in the nodular and paranodular tissue.

As in the series of patients reported previously, basal adenylate cyclase activity was significantly higher in the benign "cold" nodules and was as responsive to TSH as that of the normal tissue (Table VI). There was no significant difference in basal adenylate cyclase

TABLE VI

Adenylate Cyclase Activity in Homogenates

Patient	Diagnosis	Normal		Tumor	
		Basal	TSH	Basal	TSH
		pmol cyclic AMP formed/μg DNA/10 min			
Su	Follicular adenoma	0.16±0.02	0.3±0.03	0.5±0.02	3.2±0.03
Ha	Nodule in multinodular gland	0.19±0.01	0.4±0.02	0.5±0.01	1.8±0.04
Ju	Follicular adenoma	0.6±0.02	1.3±0.02	1.0±0.01	2.4±0.01
Ch	Embryonal adenoma	0.08±0.02	0.3±0.03	1.2±0.04	3.2±0.13
Mo	Follicular adenoma	0.4±0.03	1.5±0.03	0.9±0.03	4.8±0.04
Un	Follicular adenoma	0.6±0.04	1.7±0.08	0.3±0.08	1.1±0.03
An	Follicular adenoma	0.2±0.01	0.9±0.04	0.17±0.01	0.3±0.02
Fl	Follicular adenoma	0.4±0.05	1.1±0.05	0.7±0.08	2.4±0.02
Am	Follicular adenoma	0.2±0.03	0.6±0.04	0.5±0.11	2.0±0.03
Om	Hürthle cell adenoma	0.15±0.01	0.3±0.02	0.3±0.01	1.3±0.03
Mean ±SEM		0.3±0.06	0.8±0.2	0.6±0.1	2.3±0.4
Wi	Papillary carcinoma	0.5±0.01	1.2±0.03	0.5±0.02	1.3±0.05
He	Papillary carcinoma	0.13±0.01	0.4±0.02	1.0±0.02	1.2±0.05
Le	Papillary carcinoma	0.2±0.02	0.6±0.05	0.12±0.02	0.17±0.05
Maf	Papillary carcinoma	0.8±0.01	2.4±0.06	2.7±0.08	15.5±0.17
Mean ±SEM		0.4±0.15	1.15±0.45	1.08±0.6	4.5±3.7

The values are the mean ±SEM of triplicate determinations. TSH concentration was 1 mU/ml. The mean DNA content of normal thyroid and tumor was 3.4±0.4 μg/mg wet wt (range 1.4–6.5) and 4.2±0.6 μg/mg wet wt (range 1.7–8.6), respectively.

activity between the benign and malignant nodules. In 2 of the 4 malignant nodules, TSH stimulated adenylate cyclase activity less than 50% whereas such stimulation exceeded 75% in all 10 of the benign nodules. Although the mean data suggests that the adenylate cyclase activity in the malignant nodules was as responsive to TSH as in the benign lesions, the value is distorted by the results obtained in patient Maf.

DISCUSSION

The above data indicate that tissue slices obtained from "cold" thyroid nodules do not concentrate iodide in vitro. However, despite the defect in iodide transport, the organification of iodine and, in a smaller series, the rate of synthesis of MIT and DIT were only moderately slower in the nodules than in the surrounding normal tissue. The moderate reduction in the quantity of iodine organified in these tissues probably reflects decreased uptake of iodide from the media during in vitro incubation. Inhibition of transport in normal human thyroid with perchlorate decreased the basal rate of organification to 45% of control, but the process was still stimulated normally by TSH. Similar observations in isolated thyroid cells have been reported by Tong (18). However, since these nodules are "cold" by in vivo radioiodine scan, the amounts of iodide organified in vivo must be considerably less than in the surrounding normal tissue. The low T_4 content of these nodules also suggests an in vivo defect in organification. The apparent discrepancy between the in vivo and in vitro observations is explained if one postulates that in vivo iodide transport is rate limiting for the synthesis of thyroid hormone whereas in vitro it is not. Evidence for this is seen in the high inorganic iodide content of the normal thyroid tissues incubated for 90 min. This component accounts for over 90% of the radioactivity present in such tissues. Thus, an inability to concentrate iodide appears to be the primary defect in iodine metabolism in the "cold" nodules.

Whereas these "cold" nodules and those reported by DeGroot (7) have all shown decreased iodide transport, both benign and malignant tumors have been described in which the ability to concentrate iodide appeared to be preserved although the organification of radioiodine by these tumors could not be demonstrated (19–21). Thus, since it would appear to be generally true that "cold" thyroid nodules are "cold" because they cannot concentrate radioiodine, transport ability may be preserved and the capacity for organification lost in some thyroid tumors.

Although it has generally been well accepted that thyroid carcinomas are less efficient than normal thyroid in concentrating and organifying iodide (22), there was no consistent difference between organification in benign or malignant tissue. However, even though the number of cases is small, there was a striking difference in the response of organification to TSH in these two tissues. TSH augmented organification in slices from normal thyroid and benign "cold" nodules to about the same extent. In contrast, TSH did not stimulate organification in three of the four carcinomas in which it was studied, and the response in the fourth was small. A decrease in responsiveness to TSH was also suggested by the results obtained measuring adenylate cyclase activity. Although such data could be explained by decreased binding of TSH to the cells of some thyroid carcinomas, other possibilities exist.

The cause of the defective iodide transport in "cold" nodules is unknown. Since the biochemical basis for concentration of iodide in normal thyroid tissue has not been elucidated, characterization of the defect in the "cold" nodules is difficult. The role of an ouabain-sensitive, Na^+-K^+-activated ATPase in trapping is controversial (16, 17). Although inhibition of this enzyme by ouabain inhibited transport, the studies of Brunberg and Halmi failed to demonstrate a stoichiometric relationship between enzyme activity and iodide transport in vivo (17). Even if an ouabain-sensitive Na^+-K^+-activated ATPase is intimately associated with iodide accumulation, its activity was certainly not diminished in nodule tissue. Since the assays were done on whole homogenates, they do not provide any information concerning enzyme activities in different compartments of the cell. In general, ATPase is a membrane-associated enzyme (23). Since the fraction of total or ouabain-sensitive, cation-activated ATPase contained in the plasma membrane fraction is unknown, it is possible that changes in this fraction would be missed when the whole homogenate is assayed.

Iodide transport is energy-dependent (15), and the defect in the nodules could reflect an abnormality in production or coupling of energy for this process. An abnormality in energy production seems most unlikely since ATP concentrations in the nodules were not reduced (Table V). Furthermore, ^{32}P incorporation into phospholipids (24) and colloid droplet formation (25), both energy-requiring processes, was not diminished in slices from the "cold" nodules (1). Although the concentrating defect could reflect a block in the utilization of ATP for this process, it is also possible that the membrane carrier for iodide transport is deficient. The chemical nature of this proposed carrier is not known, although involvement of phospholipids has been suggested (26). Deletion of such a substance in neoplastic tissue would not be unique since malignant cells have sometimes been characterized by absence of enzymes (27).

Pochin has emphasized that the metabolic defects, which have been described in thyroid carcinomas, are similar to the defects that have been observed in patients with congenital goiter (*22*). The present results would extend this concept to include benign thyroid tumors as well. It is hoped that continued study of such "nonfunctioning" tissues will provide further insights into the biochemical processes involved in normal thyroid tissue.

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REFERENCES

1. DeRubertis, F., K. Yamashita, A. Dekker, P. R. Larsen, and J. B. Field. 1972. Effects of thyroid-stimulating hormone on adenyl cyclase activity and intermediary metabolism of "cold" thyroid nodules and normal human thyroid tissue. *J. Clin. Invest.* **51**: 1109.
2. Wilson, B., E. Raghupathy, T. Tonoue, and W. Tong. 1968. TSH-like actions of dibutyryl-cAMP on isolated bovine thyroid cells. *Endocrinology.* **83**: 877.
3. Ahn, C. S., and I. N. Rosenberg. 1970. Iodine metabolism in thyroid slices: effects of TSH, dibutyryl cyclic 3′,5′-AMP, NaF and prostaglandin E_1. *Endocrinology.* **86**: 396.
4. Ahn, C. S., and I. N. Rosenberg. 1968. Prompt stimulation of the organic binding of iodine in the thyroid by adenosine 3′,5′-phosphate in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **60**: 830.
5. Dumont, J. E. 1971. The action of thyrotropin on thyroid metabolism. *Vitam. Horm.* **29**: 287.
6. Larsen, P. R., F. DeRubertis, K. Yamashita, A. Dekker, and J. B. Field. 1972. In vitro demonstration of iodide trapping defect but normal thyrotropin (TSH) responsiveness in benign and malignant "cold" thyroid nodules. *Trans. Assoc. Am. Physicians Phila.* **85**: 309.
7. DeGroot, L. J. 1970. Lack of iodide trapping in "cold" nodules. *Acta Endocrinol. Panam.* **1**: 27.
8. Larsen, P. R., K. Yamashita, A. Dekker, and J. B. Field. 1973. Biochemical observations in functioning human thyroid adenomas. *J. Clin. Endocrinol. Metab.* **10**: 1009.
9. Inoue, K., and A. Taurog. 1967. Digestion of ^{131}I-labeled thyroid tissue with maximum recovery of ^{131}-iodothyronines. *Endocrinology.* **81**: 319.
10. Larsen, P. R., J. Dockalova, D. Sipula, and F. M. Wu. 1973. Immunoassay of thyroxine in unextracted human serum. *J. Clin. Endocrinol. Metab.* In press.
11. Ohta, M., R. J. Jarrett, and J. B. Field. 1966. Measurement of ATP in tissues with the use of $C^{14}O_2$ production from glucose-1-C^{14}. *J. Lab. Clin. Med.* **67**: 1013.
12. Stanbury, J. B., J. V. Wicken, and M. A. Lafferty. 1969. Preparation and properties of thyroid cell membranes. *J. Membrane Biol.* **1**: 459.
13. Zor, U., T. Kaneko, I. P. Lowe, G. Bloom, and J. B. Field. 1969. Effect of thyroid-stimulating hormone and prostaglandins on thyroid adenyl cyclase activation and cyclic adenosine 3′,5′-monophosphate. *J. Biol. Chem.* **244**: 5189.
14. Burton, K. 1955. The relation between the synthesis of deoxyribonucleic acid and the synthesis of protein in the multiplication of bacteriophage T2. *Biochem. J.* **61**: 473.
15. Tyler, D. D., J. Gonze, F. Lamey, and J. E. Dumont. 1968. Influence of mitochondrial inhibitors on the respiration and energy-dependent uptake of iodide by thyroid slices. *Biochem. J.* **106**: 123.
16. Wolff, J., and N. S. Halmi. 1963. Thyroidal iodide transport. V. The role of Na^+-K^+-activated, ouabain-sensitive adenosinetriphosphatase activity. *J. Biol. Chem.* **238**: 847.
17. Brunberg, J. A., and N. S. Halmi. 1966. The role of ouabain-sensitive adenosine triphosphatase in the stimulating effect of thyrotropin on the iodide pump in the rat thyroid. *Endocrinology.* **79**: 801.
18. Tong, W. 1964. Thyrotropin stimulation of thyroxine synthesis in isolated thyroid cells treated with perchlorate. *Endocrinology.* **75**: 968.
19. Valenta, L. 1966. Metastatic thyroid carcinoma in man concentrating iodine without organification. *J. Clin. Endocrinol. Metab.* **26**: 1317.
20. Steinberg, M., P. R. Cavaliere, and S. H. Choy. 1970. Uptake of technetium 99-Pertechnetate in a primary thyroid carcinoma: need for caution in evaluating nodules. *J. Clin. Endocrinol. Metab.* **31**: 81.
21. Usher, M. S., and A. Y. Arzoumanian. 1971. Thyroid nodule scans made with pertechnetate and iodine may give inconsistent results. *J. Nucl. Med.* **12**: 136.
22. Pochin, E. E. 1969. Thyroid adenocarcinoma: a functioning tumour. *Lancet.* **1**: 94.
23. Wolff, J., and A. B. Jones. 1971. The purification of bovine thyroid plasma membranes and the properties of membrane-bound adenyl cyclase. *J. Biol. Chem.* **246**: 3939.
24. Schneider, P. B. 1969. Effects of thyrotropin on thyroidal phospholipid and adenosine 5′-triphosphate metabolism. *J. Biol. Chem.* **244**: 4490.
25. Ahn, C. S., and I. N. Rosenberg. 1967. Proteolytic activity of the rat thyroid gland; studies using thyroid slices and subcellular fractions. *Endocrinology.* **81**: 1319.
26. Schneider, P. B., and J. Wolff. 1965. Thyroidal iodide transport. VI. On a possible role for iodide-binding phospholipids. *Biochim. Biophys. Acta.* **94**: 114.
27. Weinhouse, S. 1972. Glycolysis, respiration, and anomalous gene expression in experimental hepatomas. *Cancer Res.* **32**: 2007.