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Research Article

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The studies demonstrate an intact, TSH-responsive adenyl cyclase-cyclic AMP system in the adenomas and, accordingly, imply the presence of receptor sites for TSH on the cells of the adenoma. The failure of such nodules to concentrate ^{131}I may be owing to a subsequent impairment in the expression of cyclic AMP action on iodine metabolism.

Effects of Thyroid-Stimulating Hormone on Adenyl Cyclase Activity and Intermediary Metabolism of "Cold" Thyroid Nodules and Normal Human Thyroid Tissue

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ABSTRACT "Cold" thyroid nodules do not concentrate ^{131}I before or after thyrotropin (TSH) administration. In an attempt to elucidate the reason for this TSH unresponsiveness, the effect of TSH in vitro on several metabolic parameters was studied in 11 "cold" thyroid adenomas, 2 medullary carcinomas, and in the surrounding normal thyroid tissue. Basal adenyl cyclase activity, glucose-1-^{14}C oxidation, and ^{32}P incorporation into phospholipids were significantly greater in the adenomas than in the adjacent normal thyroid; basal cyclic 3',5'-adenosine monophosphate (cyclic AMP) concentration and adenine-^{3}H incorporation into ^{3}H-labeled cyclic AMP were not different. In adenomas as well as normal thyroid, all parameters responded significantly to in vitro TSH stimulation. The response to TSH of adenyl cyclase activity and ^{32}P incorporation was enhanced in adenomas compared with that of the adjacent normal thyroid. These differences were not explained by an increased cellularity of the adenomas. Medullary carcinomas did not respond to TSH in any of the above parameters.

The studies demonstrate an intact, TSH-responsive adenyl cyclase-cyclic AMP system in the adenomas and, accordingly, imply the presence of receptor sites for TSH on the cells of the adenoma. The failure of such nodules to concentrate ^{131}I may be owing to a subsequent impairment in the expression of cyclic AMP action on iodine metabolism.

INTRODUCTION

Nodules of the thyroid gland are a commonly encountered clinical finding with a prevalence rate of perhaps as high as 4% in the general population (1). Nonfunctioning or "cold" nodules, characterized on thyroid scintiscan by their failure to concentrate radioactive iodide both before and after thyrotropin (TSH)[1] stimulation, are of special clinical concern because of the question of malignancy which frequently leads to their surgical excision (2, 3). Although both their histologic and biochemical features have been investigated (4–6), the etiology and genesis of "cold" thyroid nodules as well as the nature of the defect in iodine metabolism remain unknown. Histologically, most of such nodules are benign and well differentiated (7, 8). A small percentage are malignant. Many are of apparent follicular cell origin and retain a potential for hormonogenesis (9, 10). Despite histologic similarity to the normal surrounding thyroid tissue, the low basal radioactive iodide accumulation of cold nodules and their unresponsiveness to TSH administration suggest the presence of significant metabolic differences. The cells of a TSH-unresponsive cold nodule might lack TSH membrane receptor sites or the defect might reflect some other abnormality of TSH cellular action. The present study was undertaken to identify, if possible, such differences by comparing the TSH responsiveness of several metabolic parameters in the nodule and the adjacent normal thyroid.

In contrast to the apparent failure of all nodules studied to concentrate ^{131}I in response to TSH in vivo, in vitro the nodules responded as well or better to TSH than the surrounding normal tissue when the adenyl cyclase-cyclic 3',5'-adenosine monophosphate (cyclic AMP) system, glucose-1-^{14}C oxidation, ^{32}P incorporation into phospholipids, and endocytosis (as seen in the electron microscope) were measured. While these studies were

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[1] *Abbreviations used in this paper:* AMP, 3',5'-adenosine monophosphate; TSH, thyroid-stimulating hormone.

in progress, DeGroot reported that such nodules were unable to concentrate ^{131}I in vitro but were able to incorporate the label into thyroglobulin (11). DeGroot's studies did not examine possible biochemical defects to account for the failure of the tissue to trap ^{131}I.

METHODS

Patients with solitary palpable thyroid nodules which did not concentrate radioactive iodine (10 μCi of Na-^{131}I orally) at 24 hr on routine scintiscan were given 10 U of TSH intramuscularly for 3 days. If on repeat thyroid scintiscan the nodule still did not concentrate ^{131}I it was classified as "cold" and non-TSH responsive. Patients with such nodules were selected for study. All patients were euthyroid by clinical and laboratory criteria (normal total serum thyroxine concentration and 24 hr ^{131}I uptake). They ranged in age from 22 to 63. 10 patients were female and 3 were male. All 13 patients were operated upon to exclude thyroid carcinoma. None were being treated with thyroid hormone preparations in the immediate period before removal of the nodule.

At surgery, the nodule as well as some surrounding thyroid tissue was obtained for comparison of several TSH-responsive metabolic functions. The excised tissue was immediately placed in normal saline at 4°C. A portion was taken for histologic examination which included an approximate estimation of cell to colloid ratio in standard hematoxylin and eosin sections of the nodule and the adjacent normal tissue. Incubation studies were begun within an hour of surgical removal. The following parameters of TSH action were compared in vitro by methods previously described: (*a*) stimulation of adenyl cyclase activity (12), (*b*) incorporation of adenine-^{3}H into ^{3}H-labeled cyclic AMP (13), (*c*) cyclic AMP concentration (14), (*d*) glucose-1-^{14}C oxidation (15), and (*e*) ^{32}P incorporation into phospholipids (16). For determination of adenyl cyclase activity (12), between 3 and 10 mg of tissue was minced and then rapidly homogenized in 0.04 M Tris HCl (pH 7.8). TSH (1 or 10 mU/ml) was added to the appropriate tubes at the beginning of the assay. The reaction mixture was incubated in air at 37°C for 10 min. All other parameters were assayed on thyroid slices weighing approximately 20 mg. Slices, prepared with a Stadie-Riggs microtome, were incubated in 25 ml Erlenmeyer flasks containing the appropriate substances in 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4). The gas phase was 95% O_2 and 5% CO_2. The flasks were incubated at 37°C in a Dubnoff metabolic shaker. The TSH concentration tested and incubation times for each parameter are as indicated in Tables I–III. Electron microscopic studies were performed on the nodules and surrounding tissue of 6 of the 13 patients. The two tumors from the patients with medullary carcinoma were not examined in the electron microscope. Tissues were incubated in Krebs-Ringer bicarbonate buffer for 1 hr. Slices were then incubated with or without TSH, 3 and 50 mU/ml, respectively, for 2 more hr and prepared for electron microscopy as previously described (17). All metabolic parameters were determined in triplicate tissue samples from the cold nodule and adjacent normal thyroid. In some instances the limited amount of tissue available prevented the study of every parameter, as shown in Tables I and II. In most experiments, however, a given metabolic parameter was assayed in both the nodule and surrounding tissue; the mean value of the triplicate determinations of the parameter in the nodule was statistically compared with the mean value in the adjacent normal tissue by Student's *t* test for paired values. Probability values were then obtained from standard tables.

RESULTS

Histology. The histologic diagnosis was follicular adenoma in seven instances, prominent hyperplastic

TABLE I

Effects of TSH In Vitro on Adenyl Cyclase Activity and Cyclic AMP Concentration in Cold Thyroid Adenomas and the Adjacent Normal Thyroid

		Cell/colloid ratio		Adenyl cyclase						Cyclic AMP					
		Normal	Adenoma	Normal			Adenoma			Normal			Adenoma		
Patient	Diagnosis			B	TSH_1	TSH_{10}	B	TSH_1	TSH_{10}	B	TSH_3	TSH_{50}	B	TSH_3	TSH_{50}
				*nmole/g per 10 min**						*nmole/g per 20 min*					
1	F. A.	2/1	10/1	—	—	—	—	—	—	1.0	6.3	7.7	0.9	29.1	41.3
2	N. M.	1/1	5/1	1.4	1.3	1.8	1.3	4.2	3.9	2.3	6.3	15.5	2.7	8.8	19.3
3	F. A.	1/1	6/2	1.4	4.6	6.3	6.1	13.3	15.1	3.0	10.1	28.0	5.8	44.0	70.6
4	F. A.	1/1	1/2	0.7	1.0	1.2	1.3	6.6	7.8	2.5	9.8	9.1	3.8	8.3	10.7
5	N. M.	—	—	0.7	1.1	1.5	2.2	7.3	9.1	1.3	8.3	9.4	1.5	10.1	19.2
6	N. M.	1/1	1/1	1.0	3.6	4.8	1.9	6.2	8.1	1.6	8.0	31.5	1.7	2.5	24.7
7	N. M.	1/1	1/2	1.3	3.5	4.8	1.6	6.6	9.8	2.2	12.3	—	4.3	9.2	—
8	F. A.	1/1	1/2	1.0	1.4	1.7	1.3	4.3	5.2	2.2	6.0	9.8	3.8	7.2	10.3
9	F. A.	1/1	1/1	2.6	5.4	6.2	3.9	17.3	21.1	3.8	9.9	22.3	2.2	24.8	37.7
10	F. A.	1/1	1/1	0.8	3.0	3.7	4.1	15.7	21.4	3.8	11.8	17.9	5.6	38.6	40.5
11	F. A.	1/1	5/1	2.4	4.5	5.3	2.7	8.0	9.4	1.6	5.6	12.2	1.5	8.8	16.7
			Mean	1.3	2.9	3.7	2.6	9.0	11.1	2.3	8.5	16.3	3.1	16.5	29.1
			SE	0.2	0.5	0.6	0.5	1.5	1.9	0.3	0.8	2.7	0.5	3.9	5.9

Incubation times for adenyl cyclase and cyclic AMP assays were 10 and 20 min, respectively. The TSH subscripts of 1, 3, 10, and 50 denote TSH concentrations of 1, 3, 10, and 50 mU/ml. Where individual values are not shown the parameter was not measured because of insufficient tissue. F. A., follicular adenoma; N. M., prominent nodule in a multinodular gland; B, basal.

* Refers to cyclic AMP formed.

TABLE II

Effects of TSH In Vitro on Certain Metabolic Parameters in Cold Thyroid Adenomas and the Adjacent Normal Thyroid

	Adenine-^{3}H incorporation into ^{3}H-labeled cyclic AMP						$^{14}CO_2$ production from glucose-^{14}C				^{32}P incorporation into phospholipids			
	Normal			Adenoma			Normal		Adenoma		Normal		Adenoma	
Patient	B	TSH_3	TSH_{50}	B	TSH_3	TSH_{50}	B	TSH_{50}	B	TSH_{50}	B	TSH_{50}	B	TSH_{50}
	pmole/g per 30 min						*cpm/mg per 45 min*				*cpm/mg per 120 min*			
1	0.3	2.6	3.1	0.2	4.4	4.6	—	—	—	—	22	38	40	77
2	1.2	4.6	7.2	2.7	10.3	10.4	52	68	150	163	108	194	150	300
3	0.5	3.1	3.8	0.3	3.0	4.5	28	36	100	132	68	129	130	543
4	1.4	7.8	11.2	0.5	6.7	6.3	7	8	33	50	49	91	63	256
5	2.5	19.3	18.1	2.2	21.7	18.2	39	71	95	170	47	130	61	282
6	2.5	12.7	—	1.7	5.7	—	—	—	—	—	—	—	—	—
7	0.9	2.2	—	3.4	7.3	—	—	—	—	—	34	79	28	70
8	4.9	9.7	20.1	1.2	2.4	2.6	—	—	—	—	113	217	147	371
9	0.7	4.0	3.7	0.7	24.7	21.4	46	63	120	212	106	255	196	336
10	0.4	2.9	4.5	0.5	15.0	17.8	74	120	134	282	90	310	126	428
11	1.9	6.6	7.7	1.5	6.5	11.2	30	36	108	144	69	132	84	302
Mean	1.6	6.9	8.8	1.4	9.8	10.8	39	58	106	165	71	158	103	297
SE	0.4	1.6	2.1	0.3	2.3	2.3	8	14	14	27	10	27	17	33

Incubation times for adenine-^{3}H incorporation into ^{3}H-labeled cyclic AMP, $^{14}CO_2$ production from glucose-1-^{14}C oxidation, and ^{32}P incorporation into phospholipids were 30, 45, and 120 min, respectively. Where individual values are not shown the parameter was not measured because of insufficient tissue. Other abbreviations are as identified in Table I.

nodule in a multinodular gland in four instances, and medullary carcinoma in two, according to the criteria of Meissner and Warren (18). The in vitro metabolic results were not measurably different in the solitary follicular adenomas and the hyperplastic nodules of multinodular glands. Neither were any differences noted with electron microscopy (four were adenomas and two nodules in a multinodular gland). Since these entities are quite similar microscopically and in addition may be related disorders (19), metabolic results were combined for purposes of statistical analysis. Both are referred to as adenomas.

Cell to colloid ratio. Cell to colloid ratio was estimated on light microscopic sections of the adenomas and the adjacent normal thyroid. Ratios varied from 2/1 to 1/1 in the normal tissue and from 1/2 to 10/1 in the adenomas (Table I).

TSH responsiveness of cold adenomas

Adenyl cyclase activity. Basal adenyl cyclase activity (Table I) was significantly greater in the adenomas than the adjacent normal thyroid (mean difference ±SE, 1.3± 0.5 nmoles cyclic AMP formed/g per 10 min, $P < 0.05$). TSH, 1 and 10 mU/ml, increased adenyl cyclase ac-

TABLE III

Effects of TSH In Vitro on Certain Metabolic Parameters in Medullary Carcinomas of the Thyroid and the Adjacent Normal Thyroid

	Adenyl cyclase		Cyclic AMP		Adenine-^{3}H into ^{3}H-labeled cyclic AMP		$^{14}CO_2$ production from glucose-^{14}C		^{32}P incorporation into phospholipids	
	Basal	TSH_{10}	Basal	TSH_{50}	Basal	TSH_{50}	Basal	TSH_{50}	Basal	TSH_{50}
	*nmole/g per 10 min**		*nmole/g per 20 min*		*pmole/g per 30 min*		*cpm/mg per 45 min*		*cpm/mg per 120 min*	
Normal	1.1±0.1	1.9±0.1	2.2±0.4	3.6±0.4	1.1±0.1	5.5±0.4	11±1	17±1	31±7	54±7
Medullary carcinoma	7.2±0.1	7.2±0.1	2.9±0.3	2.8±0.7	0.7±0.1	0.7±0.1	10±3	9±3	16±1	15±1
Normal	0.8±0.1	1.9±0.1	1.1±0.5	12.7±0.5	1.0±0.2	10 ±1.7	36±1	51±7	8±1	19±2
Medullary carcinoma	4.8±0.1	4.2±0.1	1.7±0.1	1.7±0.3	0.4±0.1	0.5±0.1	24±3	23±2	4±1	7±2

Abbreviations as in Table I. Values shown are mean ±SE of triplicate determinations.
* Refers to cyclic AMP formed.

tivity significantly in both tissues (Table I). In response to TSH, 1 mU/ml, the mean increase (±SE) was 1.6± 0.4 nmoles cyclic AMP formed/g per 10 min ($P < 0.01$) in the normal thyroid and 6.4±0.4 ($P < 0.01$) in the adenomas. Further, the adenomas appeared more responsive to TSH (Table IV) as reflected by a greater percentage increase in adenyl cyclase activity over the basal than observed in the normal tissue (mean increase ±SE in activity in the adenomas with TSH stimulation, 1 mU/ml, 145±45% greater than the increase in the normal tissue, $P < 0.02$).

Cyclic AMP concentration. Basal cyclic AMP concentration (Table I) was similar in the adenomas (3.1± 0.5 nmoles/g per 20 min) and the adjacent normal tissue (2.3±0.3). TSH, 3 and 50 mU/ml, increased cyclic AMP concentrations significantly in both tissues (Table I). In response to TSH, 3 mU/ml, the mean increase (±SE) was 6.2±0.6 nmoles/g per 20 min ($P < 0.01$) in the normal thyroid and 13.4±3.7 ($P < 0.01$) in the adenomas. Although the TSH responsiveness of the adenomas tended to be greater than that of the adjacent normal tissue in this parameter (Table IV), the difference was not statistically significant.

Adenine-^{3}H incorporation into ^{3}H-labeled cyclic AMP. Basal incorporation of adenine-^{3}H into ^{3}H-labeled cyclic AMP (Table II) was similar in the adenomas (mean ±SE, 1.4±0.3 pmoles/g per 30 min) and the normal tissue (1.6±0.4). TSH, 3 and 50 mU/ml, increased adenine-^{3}H incorporation into ^{3}H-labeled cyclic AMP significantly in both tissues (Table II). In response to TSH, 3 mU/ml, the mean increase (±SE) was 5.3±1.3 pmoles/g per 30 min ($P < 0.01$) in the normal thyroid and 8.4±2.3 ($P < 0.01$) in the adenomas. No statistically significant difference existed in the TSH responsiveness (Table IV) of the two tissues in this parameter.

TABLE IV

Thyrotropin (TSH) Responsiveness of Cold Adenomas Relative to the Adjacent Normal Thyroid

Parameter	TSH concentration	Difference in responsiveness to TSH* (adenoma-normal)	P values
	mU/ml	*% increase over basal*	
Adenyl cyclase activity	1	145 ± 45	<0.02
Cyclic AMP concentration	3	198 ±150	NS
Adenine-^{3}H incorporation into ^{3}H-labeled cyclic AMP	3	690 ±315	NS
$^{14}CO_2$ production	50	16.4± 9.8	NS
^{32}P incorporation into phospholipids	50	84.8± 33.2	<0.05

* Calculated as: $\left[\frac{\text{TSH-basal (adenoma)}}{\text{basal (adenoma)}} - \frac{\text{TSH-basal (normal)}}{\text{basal (normal)}}\right] \times 100$; mean differences ±SE are shown; P values based on paired t test.

$^{14}CO_2$ production from glucose-1-^{14}C. Basal glucose oxidation (Table II) was significantly higher in the adenomas than the adjacent normal thyroid (mean difference ±SE, 67±8 cpm/mg per 45 min, $P < 0.001$). Neither the normal thyroid nor the adenomas responded to TSH, 3 mU/ml, with a detectable change in $^{14}CO_2$ production. However, TSH, 50 mU/ml, resulted in a significant increase in $^{14}CO_2$ production in both the normal thyroid (mean increase ±SE, 19±6, $P < 0.05$) and the adenomas (59±16, $P < 0.05$). The TSH responsiveness of the adenomas (Table IV) was not statistically greater than that of the adjacent tissue.

^{32}P incorporation into phospholipids. Basal ^{32}P incorporation into phospholipids (Table II) was significantly greater in the adenomas than the surrounding normal tissue (mean difference ±SE, 32±9 cpm/mg per 120 min, $P < 0.01$). Neither the normal tissue nor the adenomas responded to TSH, 3 mU/ml, with a detectable change in ^{32}P incorporation. However, TSH, 50 mU/ml, resulted in a significant increase in ^{32}P incorporation in both the normal thyroid (mean increase ±SE, 87±19 cpm/mg per 120 min, $P < 0.01$) and the adenomas (194±35, $P < 0.001$). The TSH responsiveness of the adenomas was significantly greater than that of the adjacent tissue (Table IV) in this parameter (mean increase ±SE in ^{32}P incorporation in the adenomas with TSH stimulation, 50 mU/ml, 84.8±33.2% greater than the increase in the normal thyroid, $P < 0.05$).

Electron microscopy. In the electron microscope, the follicular cells of the nonstimulated adenomas were taller than the cells of the adjacent normal thyroid tissue (mean height ±SE, 5±0.5 μ in the normal and 13±2.1 in the adenomas, comparing height of the tallest cell per follicle in five follicles). The nuclei of the adenoma cells tended to be larger and more irregular in shape than those of the normal thyroid (mean nuclear surface area as micromicrons ±SE, 16±2.6 in the normal and 41±4.6 in the adenomas, comparing the largest nucleus per follicle in five follicles). Otherwise no detectable differences in the organelles such as mitochondria, ribosomes, Golgi complexes, endoplasmic reticulum, lysosomes, or small endocytic vesicles were apparent. The surrounding thyroid tissue generally had more lipofuscin than the adenoma.

No colloid droplets were seen in control slices of normal or adenoma tissue. In response to TSH, 3 mU/ml, colloid droplet formation was more markedly enhanced in the adenomas than in the normal surrounding tissue (Figs. 1 and 2). In the adenomas compared with normal thyroid, droplets were increased both in number (mean per cell ±SE, 1.3±0.3 in normal and 2.1±0.4 in adenomas, counting droplets per cell in five follicles) and in size (mean diameter as microns ±SE, 1.6±0.2 in normal and 2.5±0.4 in adenomas, comparing the largest droplet per

follicle in five follicles). Further, adenoma colloid droplets were less uniform in structure than those of the normal thyroid. The colloid droplets both in the normal thyroid glands and adenomas were present only in the apical portions of the cells. Lysosomes were seen, on occasion, in close proximity to colloid droplets, in both the thyroid glands and adenomas. Compared with the response of the normal thyroid to TSH (3 mU/ml), the number of pseudopods were not significantly increased in the adenomas, although these structures in the adenomas were more irregular in shape and larger in size (mean surface areas as micromicrons ±SE, 5.8±1.0 in the normal and 11.7±2.7 in the adenomas, comparing the largest pseudopod per follicle in five follicles).

Not all adenomas responded with equal intensity to TSH but all displayed greater response than the adjacent normal tissue. As with the normal thyroid controls, not all adenomatous follicles were equally responsive to TSH. However, a greater proportion of the follicles in adenomas were TSH responsive, especially the smaller adenomatous follicles. There was no correlation between the response of the biochemical parameters to TSH in the different tumors and the anatomical findings from case to case. The response of the tissues to 3 mU/ml TSH appeared to be maximal, e.g., no greater effect was seen with 50 mU/ml. No basic difference in the response or the anatomical structure of the tumors classified as adenomas or hyperplastic nodules in a nodular thyroid was seen with the electron microscope. One tumor (from patient 8) had excessive granular material within the cytoplasm which in the light microscope was periodic acid-Schiff (PAS) positive, but not resistant to diastase digestion. Therefore, the substance was considered to be glycogen, similar to that reported by Klinck (20). The metabolic responses of this tumor did not differ from the others.

TSH responsiveness of medullary carcinomas. In vitro TSH responsiveness was studied in two medullary carcinomas which did not concentrate ^{131}I on 24 hr scintiscan before and after TSH administration in vivo. Responses to the highest in vitro TSH concentrations employed are summarized in Table III. The medullary carcinomas in contrast to the adjacent normal tissue failed to respond consistently to TSH in vitro in any metabolic parameter tested, although one carcinoma demonstrated a modest increase in ^{32}P incorporation into phospholipids (4±1 cpm/mg per 120 min increasing to 7±2 with 50 mU TSH/ml).

DISCUSSION

In the normal thyroid, TSH appears to be a primary physiologic regulator of iodide transport (21), organification (22, 23), and release (24, 25). Further, these actions of TSH seem to be mediated by the adenyl cyclase-cyclic AMP system (26-28). Recent evidence has indicated that TSH-responsive adenyl cyclase is found in the plasma membrane fraction of thyroid cells (29, 30) and suggests that adenyl cyclase activation is associated with TSH interaction at the surface of the cell. Therefore, the failure of cold thyroid nodules to concentrate ^{131}I in vivo might be related to : (*a*) impaired TSH attachment to the cells of the adenoma; (*b*) a TSH-unresponsive adenyl cyclase-cyclic AMP system; or (*c*) a subsequent impairment in the expression of cyclic AMP action on iodine metabolism. The latter might be a consequence of the recently recognized abnormality in iodide trapping by such nodules (11).

The results of the present study imply that the failure of the nodules to increase iodide concentration significantly in vivo in response to TSH is not due to impaired binding of TSH to the cell membrane or subsequent activation of the adenyl cyclase-cyclic AMP system. The adenyl cyclase-cyclic AMP system of the cold adenomas was equally (cyclic AMP concentration and adenine-^{3}H incorporation into ^{3}H-labeled cyclic AMP) or more responsive (adenyl cyclase activity) to identical concentrations of TSH than the adjacent normal thyroid (Tables I, II, and IV). TSH stimulation of thyroidal glucose-1-^{14}C oxidation, ^{32}P incorporation into phospholipids, and colloid droplet formation are end-organ responses thought to be mediated by TSH enhancement of intracellular cyclic AMP concentration (31, 32). These parameters were also equally (glucose oxidation) or more responsive (^{32}P incorporation, colloid droplet formation) to in vitro TSH stimulation in the cold adenomas (Tables II and IV). Thus, the expression of a number of cyclic AMP-mediated actions of TSH appears to be intact in these adenomas. The ultimate mechanism by which cyclic AMP increases certain parameters of intermediary metabolism and their relationship, if any, to in vivo thyroidal iodine metabolism, is unknown.

The basal activity of adenyl cyclase, glucose-1-^{14}C oxidation and ^{32}P incorporation into phospholipids was significantly greater in the adenomas than in the adjacent normal tissue. The explanation for these differences in basal activity is not apparent. However, as in the instance of the enhanced TSH responsiveness of the adenomas in certain parameters, the basal differences could not be attributed to increased cellularity of this tissue (Table I). Such basal differences might be related to the larger cell size of the adenomas as seen with the electron microscope. Increased basal activity of cold adenomas was not a uniform feature of all parameters examined. Thus, accelerated basal function due to loss of a local negative feedback control mechanism or enhanced adenoma sensitivity to endogenous TSH also seemed unlikely explanations.

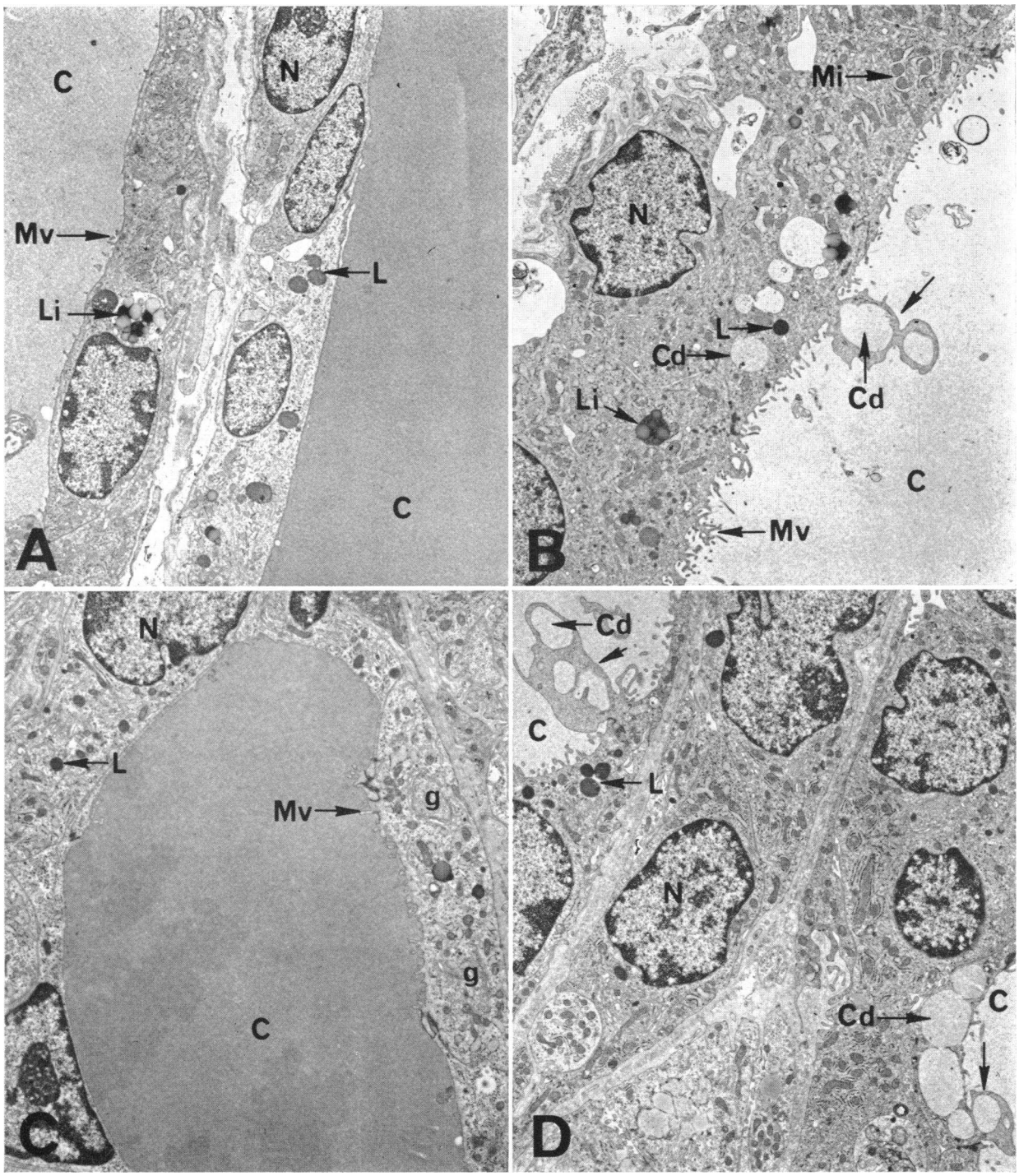

FIGURE 1 Electron micrographs comparing the response of thyroid gland (*A-B*) and adenoma (*C-D*) incubated with and without TSH (patient 3). C = colloid; g = Golgi complexes; L = lysosomes; Li = lipofuscin; Mv = microvilli; mi = mitochondrium; N = nucleus (uranyl acetate and lead citrate). *A*, thyroid gland incubated for 3 hr in Krebs-Ringer bicarbonate (KRB) buffer. Note flat epithelial cells, presence of lysosomes (L), and absence of colloid droplets. (× 4200). *B*, thyroid gland incubated for 2 hr with 3 mU/ml of TSH.

The in vitro responsiveness of the adenomas to TSH is not totally unexpected since such neoplasms are of probable follicular cell origin and may retain a potential for hormonogenesis (9, 10). Further, the growth rate of certain thyroid malignancies of follicular cell origin appears to retain some dependence on TSH. Presumed suppression of TSH by exogenously administered thyroid hormone has been reported to result in gross and radiologic evidence of tumor regression in a variety of such thyroid tumors (33). Medullary carcinomas of the thyroid in contrast, arising from parafollicular cells which secrete calcitonin, probably are not TSH responsive and have little to do with thyroid hormone biosynthesis (34). Such tumors showed no significant response to TSH in vitro (Table III) in any parameter examined in this study, confirming their independence of TSH control.

From the present data, it would appear that the impaired in vivo iodide uptake of cold adenomas could involve a metabolic block between the generation of cyclic AMP and the stimulation of those parameters directly related to iodide concentration. An in vivo iodide-concentrating defect, as reflected by the static measurement provided by a thyroid scintiscan, might depend on a complex alteration in the kinetics of one or more steps (trapping, organification, release) of iodine metabolism in these nodules. However, DeGroot has recently reported that cold nodules failed to trap ^{131}I in vitro. Such nodules were unable to maintain a normal tissue slice-medium ^{131}I gradient in the presence of methimazole but were able to incorporate the label into thyroglobulin in the absence of this blocking agent (11). An isolated defect in nodule iodide transport was suggested, perhaps qualitatively similar to that in congenital goitrous hypothyroidism (35). DeGroot did not investigate possible biochemical defects to explain the impaired iodide trapping. The results of the present study, however, by demonstrating an intact, TSH-responsive adenyl cyclase-cyclic AMP system in such nodules, would be consistent with the presence of a metabolic block preventing expression of cyclic AMP-mediated iodide trapping. Studies are currently in progress in this laboratory to characterize more precisely, if possible, the nature of the biochemical defect responsible for impaired iodide transport in such nodules.

Mechanisms to account for the reduced in vivo iodine concentration by cold nodules other than abnormal iodide trapping have also been investigated (36–39). However, no specific biochemical abnormality has been identified. Further, there is evidence against several possible mechanisms including: (*a*) a reduction in the thyroglobulin concentration of cold nodules (6); (*b*) a primary physicochemical abnormality in the thyroglobulin of cold nodules which might prevent normal iodination (37); and (*c*) enhancement of protease (38) or dehalogenase activity (39) which might accelerate iodide release.

Klinck, Oertel, and Winship (40) reported the infrequent occurrence of colloid droplets in unstimulated, normal human thyroid tissue, which is confirmed in this study. However, it is apparent that in both normal and well-differentiated neoplastic thyroid tissues (adenomas), the basic endocytic response to in vitro TSH stimulation is similar to that seen in animals (17, 41). As a matter of fact, the exaggerated response in the adenomas correlates well with the biochemical findings. The temporal

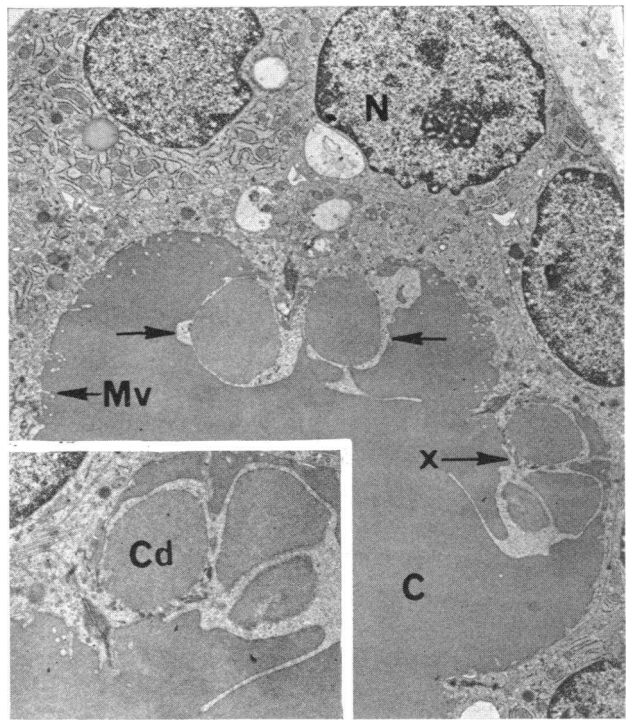

FIGURE 2 Adenoma incubated for 2 hr with 3 mU/ml of TSH (patient 10). Note prominent and bizarre pseudopods (free arrows) containing large colloid droplets (Cd). N = nucleus; Mv = microvilli; C = colloid. (Uranyl acetate and lead citrate, × 5880.) *Inset,* higher magnification of area indicated by x. (× 9660).

(*Fig. 1 continued*)

Note pseudopod (free arrow) with prominent colloid droplets (Cd) in the cytoplasm. Microvilli (Mv) also appear to be more prominent. (× 4200). *C,* adenoma incubated for 3 hr in KRB buffer. Note flat overlapping epithelial cells and absence of endocytosis. (× 5880). *D,* adenoma incubated for 2 hr with 50 mU/ml of TSH. Note prominent pseudopods (free arrows) and large colloid droplets (Cd). (× 5880).

sequence of the endocytic process in the 2 hr incubation period of this study appears to be similar to that reported in animals (42).

An effort is usually made by pathologists to differentiate adenomas from hyperplastic nodules occurring in nodular thyroids or goiters. This study has shown the essential metabolic and anatomic similarity between these two entities and points out that their similarity should be stressed rather than their differences. Of course, the anatomical differentiation of follicular adenomas from well-differentiated follicular carcinoma remains challenging (18).

In summary, the results of the present study imply that defective iodide uptake in cold follicular adenomas is not likely related to an absence of cellular TSH receptor sites. The presence of a TSH-responsive adenyl cyclase-cyclic AMP system as well as intact cellular expression of many of the actions of TSH considered to be mediated by cyclic AMP was demonstrated in vitro in these cold nodules. Since the effects of TSH on thyroidal iodide metabolism also appear to be mediated by cyclic AMP, the failure of TSH to increase significantly nodule iodide uptake in vivo may be related to a subsequent impairment in the expression of cyclic AMP action on iodine metabolism. The specific biochemical defect responsible, however, remains to be elucidated.

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