

# COLCHICINE-BINDING PROTEIN AND THE SECRETION OF THYROID HORMONE

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## ABSTRACT

The role of microtubules in the thyrotropin- or adenosine 3',5' cyclic monophosphate (cyclic AMP)-stimulated accumulation of cytoplasmic colloid droplets and secretion of iodine from the mouse thyroid gland has been investigated by means of different classes of agents that affect the stability of microtubules. The onset of inhibition of secretion by colchicine, the uptake of colchicine-<sup>3</sup>H by thyroid lobes, and the binding of colchicine-<sup>3</sup>H to thyroidal soluble protein are shown to have similar time courses. Colloid droplet accumulation is also inhibited and does not readily resume upon removal of colchicine from the medium. This appears to be due to the slow washout of the drug ( $t_{1/2} \sim 7$  hr). Thyroids contain a soluble colchicine-binding protein that resembles microtubule proteins of other tissues with respect to apparent  $K_m$  for colchicine, pH optimum, and stability characteristics. Colchicine analogues inhibit iodine secretion and colchicine binding in a parallel manner and as a function of their antimetabolic potencies. Microtubule-stabilizing agents such as hexylene glycol and D<sub>2</sub>O also inhibit secretion. Thus, inhibition of thyroid secretion by antimetabolic agents appears to be mediated by an effect on microtubules. The inhibitory locus of colchicine inhibition occurs after the generation of cyclic AMP, since stimulation of secretion by this nucleotide is blocked by colchicine, whereas thyroid-stimulating hormone-induced accumulation of cyclic AMP is not affected. Thus, the functioning microtubule appears to play a role in the induction of colloid endocytosis.

## INTRODUCTION

The bulk of the thyroid hormone and organic iodine present in thyroid glands is stored in the follicular lumen of the gland in the form of 19S thyroglobulin (mol wt  $\sim 670,000$ ) and related proteins of both smaller (12S) and greater (27-37S) size (1). On the other hand, the bulk of the circulating organic iodine exists in the form of iodo-amino acids derived hydrolytically from the above iodinated proteins. Although other pathways of hormone secretion have not been ruled out, the present concept for activation of thyroid secretion

by thyroid-stimulating hormone (TSH)<sup>1</sup> involves the following steps (a) TSH combines with a membrane receptor and activates the membrane-bound adenylate cyclase (2, 3). (b) The increased intra-

<sup>1</sup> *Abbreviations used include:* cyclic AMP, adenosine 3',5' cyclic monophosphate; dibutyryl cyclic AMP, N<sup>6</sup>-2-O-dibutyryl-adenosine 3',5' cyclic monophosphate; SPMG, 0.25 M sucrose, 10 mM Na phosphate, pH 7.0, 10 mM MgCl<sub>2</sub>, and 0.1 mM GTP solution; PMG, the same buffer without sucrose, TSH, thyroid-stimulating hormone.

cellular level of adenosine 3',5' cyclic monophosphate (cyclic AMP) then activates colloid endocytosis. (c) This is followed by fusion of the colloid droplets with lysosomes, intracellular digestion, and release of thyroid hormone (4). We have recently shown that colchicine, and other agents that can disaggregate microtubules, inhibit TSH- and N<sup>6</sup>-2-O-dibutyryl-adenosine 3',5' cyclic monophosphate (dibutyryl cyclic AMP)-stimulated colloid droplet formation and <sup>131</sup>I release. We therefore suggested a role for microtubules in thyroid secretion (5). The present work extends this proposal and provides further evidence that colchicine-induced inhibition of thyroid hormone release is caused by an effect on microtubular protein and that the microtubule-dependent step occurs beyond the stage of cyclic AMP generation.

#### METHODS

Thyroid secretion was studied *in vitro* by use of the mouse thyroid system previously described (6). Thyroid glands were labeled with <sup>131</sup>I for 2 hr *in vivo*, removed on the trachea, and incubated in Earle's solution for 4-6 hr. Where specified, glands were preincubated for 2 hr with colchicine or an analog and were then transferred to a new flask containing the same concentration of the agent and TSH or cyclic AMP. The fraction of the total thyroidal <sup>131</sup>I released was calculated from the radioactivity of the thyroid and that of an aliquot of the medium after incubation.

Colloid droplets were counted using previous criteria (7) in thyroids fixed in Bouin's solution, embedded in paraffin, and sectioned at 6  $\mu$ . Values are reported either as the number of droplets per 50 follicles or as the number of droplets per 100 nuclei (25 follicles were counted in the latter case).

Thyroidal cyclic AMP levels were determined using the protein binding method of Gilman (8) modified in that thyroids were homogenized in 50% glacial acetic acid, centrifuged, and cyclic AMP content was determined in aliquots of the supernatant solution after drying. For analysis of cyclic AMP, thyroids were preincubated on the trachea for 2 hr as in secretion experiments, and the thyroid lobes were then removed, pooled, and weighed on a torsion balance. Lobes from two or three mice per flask were then incubated 1 hr in similar medium with TSH as specified. The thyroids were then quickly homogenized in 300  $\mu$ l of cold 50% glacial acetic acid and the homogenate was centrifuged for 1 min in a Beckman Microfuge (Beckman Instruments, Inc., Fullerton, Calif.). Aliquots of 20-100  $\mu$ l were dried at 80°C and assayed in duplicate for

cyclic AMP against a standard curve run simultaneously. We would like to thank Dr. Eric Gruenstein for his help in these determinations.

The uptake of colchicine-<sup>3</sup>H by mouse thyroid glands was studied by incubating the thyroid on the trachea in Earle's solution containing 0.5  $\mu$ Ci/ml colchicine-<sup>3</sup>H at the specified concentration of colchicine. After incubation for times varying from 15 min to 6 hr, the tissue was swirled for 30 sec in a large volume of Earle's solution. The thyroid lobes were then dissected free, weighed on a torsion balance, and dissolved in 0.5 ml of Soluene (Packard Instrument Co., Downers Grove, Ill.) in a scintillation vial. 15 ml of Bray's solution were then added and the radioactivity was determined. Quenching was corrected for by use of an internal standard and results are expressed as picomoles of colchicine per milligram of thyroid tissue.

Binding of colchicine-<sup>3</sup>H to soluble thyroid protein was studied using the 100,000 g (1 hr) supernatant solution of ovine thyroid gland homogenized in 3 vol of a solution of 0.25 M sucrose, 10 mM Na phosphate, pH 7.0, 10 mM MgCl<sub>2</sub>, and 10<sup>-4</sup> M guanosine triphosphate (GTP) (SMPG) at 2°C (5). Pellets contained less than 10% of the total colchicine bound by crude homogenates and were not studied. With pooled mouse thyroid glands, homogenate concentrations of 100 mg of tissue per ml of medium were used. The supernatant solution was either used directly or frozen at -30°C. There was no loss of activity after 1 wk. Usually 0.45 ml of the SMPG thyroid supernatant solution was incubated at 37°C with 0.05 ml of H<sub>2</sub>O containing 2.5  $\mu$ Ci/ml of colchicine-<sup>3</sup>H and 10 times the desired colchicine concentrations. After incubation, tubes were cooled rapidly to 0°C and protein-bound colchicine was assayed by the DEAE cellulose filter disc method of Weisenberg et al. (9). For consistent results, two superimposed filter discs were used. The discs were prepared by passing 2.5 ml of 10 mM phosphate buffer, pH 7.0, with 10 mM MgCl<sub>2</sub> and 10<sup>-4</sup> M GTP (PMG) through the filters with mild suction, being careful not to dry the filters by prolonged suction. The degree of wetting determines the subsequent filtration rate, and had to be adjusted such that the time required for subsequent filtration by gravity was > 8 min and < 30 min. Successive additions to the filter chimneys were 1 ml of PMG with 1·10<sup>-5</sup> M colchicine, 0.1-0.2 ml of sample, and 4 ml of PMG with 10<sup>-5</sup> M colchicine, all at 2°C. After gravity filtration, filters were washed four times with 4.0 ml of PMG at 2°C by suction and counted in 6 ml of Bray's solution. Background binding of free colchicine was determined by assaying a similar aliquot of SMPG containing colchicine-<sup>3</sup>H but no protein; this usually was 0.05-0.10% of the total radioactivity present. In the presence of colchicine-binding protein, 5-8% of labeled colchicine was

bound to the filters. The background was relatively important only at low activities or when binding was markedly decreased. The coefficient of variation on 10 aliquots from the same sample assayed simultaneously was 1.2%. Results are expressed as picomoles of colchicine bound per milliliter since the protein of the supernatant solution consisted primarily of thyroglobulin and varied between 20 and 40 mg/ml. Purified thyroglobulin itself does not bind colchicine.

## RESULTS

### *Time Course of Colchicine-Induced Inhibition of Thyroid Secretion*

In an earlier report, we found that the inhibitory effect of colchicine on iodine secretion showed an inverse time-concentration relationship. Thus, low concentrations of colchicine required 2-4 hr of preincubation to produce maximum inhibitory effects, whereas immediate effects occurred only with high concentrations (5). The slow onset of colchicine effects, and the importance of preincubation, are depicted in Fig. 1. With  $1 \cdot 10^{-5}$  to  $1 \cdot 10^{-4}$  colchicine, rapid inhibition of iodine secretion can be obtained but such inhibition is incomplete. Even  $1 \cdot 10^{-8}$  colchicine does not abolish the formation of colloid droplets (see below) when added simultaneously with TSH. To obviate possible nonspecific effects such high concentrations were not used.

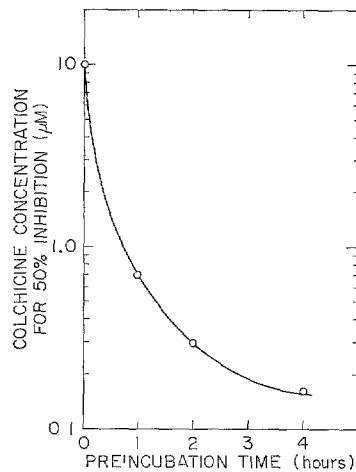


FIGURE 1 The time-concentration dependence of colchicine inhibition of thyroid secretion. The colchicine concentrations required to inhibit by 50% the TSH-stimulated release of  $^{131}\text{I}$  from prelabeled mouse thyroids were obtained from dose-inhibition curves at various preincubation times (5).

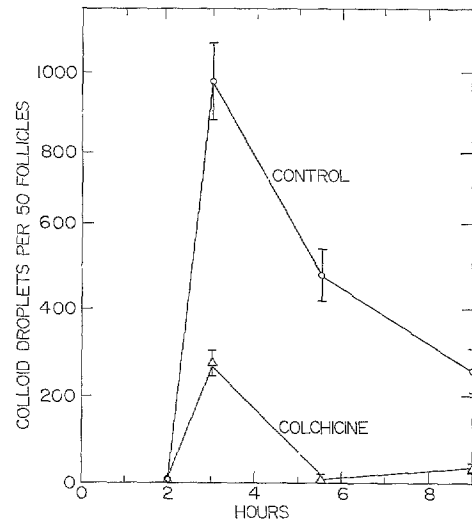


FIGURE 2 Failure of reversal of colchicine inhibition of TSH-stimulated colloid droplet accumulation. The response to a 1 hr exposure to 2.5 munits/ml of TSH, added at 2, 4.5, or 8 hr, was determined.  $\Delta = 5 \cdot 10^{-6}$  M colchicine present for the first 3 hr and absent thereafter.  $\circ$  = control medium without colchicine. Preincubation was for 2 hr, and the 2 hr point represents the baseline droplet count. Values are the means  $\pm$  SE of three thyroids per group.

Once mouse glands have been exposed to colchicine, subsequent incubation in colchicine-free media does not reverse the inhibitory state for periods up to 6 hr as shown in Fig. 2. Here the colloid droplet response to TSH is used as an index of secretion. After 2 hr of preincubation the thyroids contained very few colloid droplets in either control or colchicine-treated tissues. The response to TSH was markedly attenuated by  $5 \cdot 10^{-6}$  M colchicine (3-hr points). Incubation was continued without colchicine, but despite this, glands that had been treated with colchicine continued to be unable to respond to TSH for as long as 5 hr (8 hr point). In fact, inhibition was more nearly complete at the later intervals. The decrease of the droplet response occurring with time in the control curve was variable and did not occur in some experiments. The reason for this variability is not known. This poor reversal of the colchicine effect is in accord with findings on other systems (11-13).

In contrast to the above findings, once the TSH-stimulated response had been initiated, the inhibitory effect of colchicine was slow to manifest itself. This is depicted in Fig. 3 where the colloid droplet response is shown when colchicine was

added *after* TSH. At a time when inhibition should have been complete if colchicine had been added *before* TSH (Fig. 2) (5), the droplet count was reduced by only about 50% in the presence of  $1 \cdot 10^{-5}$  M colchicine. Thus, inhibition of a secretory response already initiated by TSH requires more time than that required to block initiation of endocytosis.

The possibility had to be considered that the slow onset of inhibition produced by colchicine was due to either a slow rate of penetration or a slow rate of binding of the drug (14). Fig. 4 shows data for the uptake and washout of colchicine- $^3$ H by mouse thyroid glands *in vitro*. The colchicine uptake curves (Fig. 4 A) are similar to those obtained with beef thyroid slices (5), except that steady-state colchicine concentrations are higher. Mouse thyroids rapidly (30 min) attain the colchicine concentrations of the surrounding medium (indicated by horizontal bars) and then accumulate the drug more slowly. In separate experiments the fraction of total mouse thyroid colchicine that was bound to protein during the first 15–60 min amounted to  $\sim 25\%$  when the external concentration was  $5 \cdot 10^{-6}$  M. As in the case of KB cells (14), the rate of attaining equilibrium is concentration dependent.

The washout of colchicine- $^3$ H from equilibrated mouse thyroid glands into colchicine-free media shows at least two components (Fig 4 B). The slow component has a half-life of  $\sim 7$  hr. A bi-

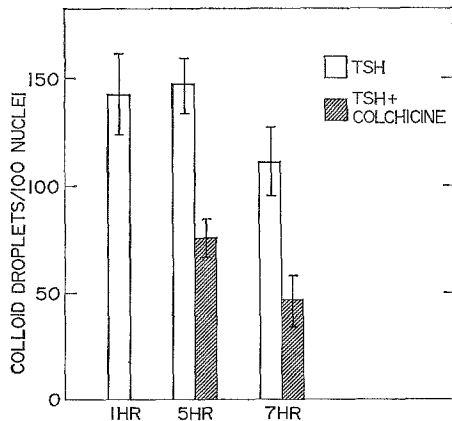


FIGURE 3 Effect of colchicine after induction of colloid droplet accumulation by TSH.  $1 \cdot 10^{-5}$  M colchicine was added 1 hr after 2.5 munits/ml of TSH (shaded bars). The 1 hr value depicts the standard TSH response before colchicine was added. Each point represents the mean  $\pm$ SE of four mice.

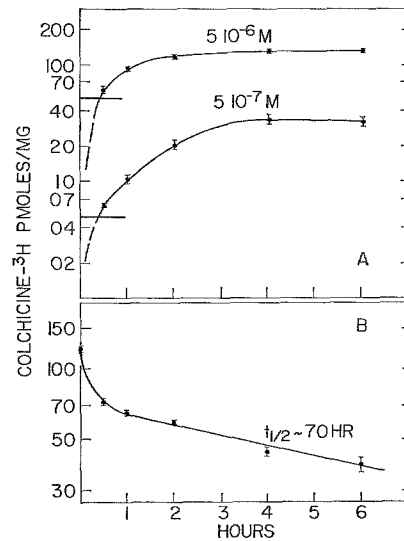


FIGURE 4 Uptake and washout of colchicine- $^3$ H by mouse thyroid glands *in vitro*. (A) Uptake of colchicine- $^3$ H at intervals ranging from 15 min to 6 hr from medium containing  $5 \cdot 10^{-7}$  M or  $5 \cdot 10^{-6}$  M colchicine. (B) Amount of colchicine- $^3$ H remaining in thyroids incubated 2 hr in  $5 \cdot 10^{-6}$  M colchicine- $^3$ H and transferred at time zero to colchicine-free media. All values are the mean  $\pm$ SE for three thyroids. The horizontal lines indicate the colchicine concentration of the medium (assuming specific gravities of 1.0).

phasic loss of colchicine has also been shown in sea urchin eggs (15). Extrapolation and subtraction of the slow component from the early time points suggests the presence of a rapidly lost component which may represent the unbound colchicine- $^3$ H. It is approximately equal to that which would be present in the tissue water at the concentration of the loading medium (see Fig. 4 A). Thus, both inward and outward movement of free colchicine across the thyroid cell membranes occurs rapidly. The slower phase of uptake and release of colchicine is most likely related to the slow rate of binding (see below) and may explain a part of the slow onset and reversal of colchicine-induced inhibition of secretion. However, at some concentrations (e.g.  $5 \cdot 10^{-6}$  M), the temporal discrepancy between binding of colchicine and inhibition of secretion (compare Figs. 4 A and 1) suggests that other factors must also play a role.

#### Localization of the Colchicine Inhibition

Colchicine blocks the stimulating action of dibutyryl cyclic AMP on  $^{131}$ I release while adenylyl

TABLE I  
Effects of Colchicine on Cyclic AMP-Stimulated  $^{131}\text{I}$  Release by Mouse Thyroids In Vitro

Colchicine	Per cent of thyroidal $^{131}\text{I}$ release		
	Basal	3 mM cyclic AMP	10 mM cyclic AMP
<i>M</i>			
None	2.7 ± 0.9	20.0 ± 1.2	27.7 ± 0.8
2 × 10 <sup>-7</sup>	1.6 ± 0.3	15.4 ± 1.1 (20%)	22.6 ± 0.5 (16%)
2 × 10 <sup>-6</sup>	2.9 ± 0.6	6.0 ± 0.6 (82%)	11.8 ± 1.3 (65%)

Thyroids were labeled in vivo for 2 hr with  $^{131}\text{I}$ , preincubated in vitro for 2 hr with colchicine at the specified concentration, and incubated in similar media with cyclic AMP added as noted. The value in parentheses is the per cent of inhibition calculated by comparing the cyclic AMP-stimulated increment in  $^{131}\text{I}$  release to that found without colchicine. All values are means ± SE of three to five thyroids.

TABLE II  
The Effect of Colchicine on Basal and TSH-Stimulated Cyclic AMP Levels in Mouse Thyroids In Vitro

Colchicine	Cyclic AMP			
	Basal	Colchicine	TSH	TSH + colchicine
<i>M</i>				
1 × 10 <sup>-5</sup>	0.8 ± 0.2	1.1 ± 0.3	6.6 ± 1.0	6.6 ± 1.1
1 × 10 <sup>-4</sup>	0.6 ± 0.1	0.8 ± 0.2	6.2 ± 0.8	8.2 ± 1.5

Thyroids were preincubated in Earle's solution with colchicine for 2 hr and then incubated in similar media with 2.5 munits/ml TSH for 1 hr. All values are the means ± SE of three or four flasks containing two or three mouse thyroids.

cyclase and phosphodiesterase activities are not affected (5). Since dibutyl cyclic AMP can function as a phosphodiesterase inhibitor (16), and since the mouse thyroid gland responds well to cyclic AMP (6, 17), the effect of colchicine on cyclic AMP-stimulated  $^{131}\text{I}$  release was studied. As shown in Table I, colchicine inhibited the action of cyclic AMP on  $^{131}\text{I}$  secretion. There was slightly less inhibition at the higher concentration of cyclic AMP. Thus, the inhibitory locus for colchicine must lie after the generation of cyclic AMP.

Since measurement of adenylate cyclase activity does not necessarily reflect tissue levels of cyclic AMP, it was important to know whether or not colchicine could influence these levels in thyroid tissue. It is known that TSH increases tissue levels of cyclic AMP (18, 19). As shown in Table II, TSH produced an 8- to 10-fold increase in the cyclic AMP concentration of mouse thyroids stimulated in vitro. Preincubation in large concen-

trations of colchicine, which markedly inhibit the subsequent secretory or colloid droplet response (Fig. 2), did not influence the response of the cyclic AMP levels to TSH.

#### Properties of Colchicine-Binding Protein

If colchicine inhibition of thyroid secretion is mediated through binding to microtubular protein, it should be possible to correlate effects on this process with effects on  $^{131}\text{I}$  release. Binding of colchicine- $^3\text{H}$  to a 6S thyroidal protein has been demonstrated (5). Binding of colchicine- $^3\text{H}$  can also be demonstrated using the high speed supernatant of thyroid homogenates. The binding of colchicine as a function of time and concentration at 37°C is shown in Fig. 5. There was no significant binding at 2°C. The slow approach to the steady state, particularly at low colchicine concentrations, resembles the uptake of colchicine- $^3\text{H}$  and the on-

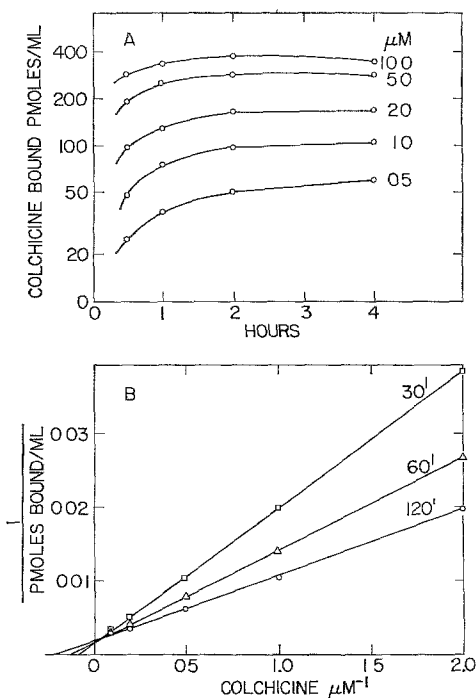


FIGURE 5 (A) Colchicine-<sup>3</sup>H binding to soluble protein from beef thyroid as a function of time for various colchicine concentrations. (B) Double reciprocal plot of bound colchicine vs. medium concentration for 30, 60, and 120 min taken from the data of Fig. 6 A.

set of secretory inhibition observed in intact thyroid glands (Figs 1, 3) (5) Reciprocal plots of steady-state amounts of bound colchicine vs. concentration reveal an apparent  $K_m$  of  $2-4 \cdot 10^{-6}$  M in several experiments. It is unlikely, however, that this binding system obeys simple first order kinetics (with respect to colchicine) since apparent  $K_m$  values calculated for shorter binding times are as great as  $1 \cdot 10^{-5}$  M (Fig. 5 B). The relatively rapid decay of the binding site (see Fig. 6) and the supposed protection of colchicine-binding proteins by colchicine may well account for this kinetic behavior.

The pH optimum of colchicine binding to the high speed supernatant solution of beef thyroid glands ranged from 6.7 to 7.1 in phosphate, imidazole, or Tris-maleate buffers. This optimum is like that reported by Wilson for the binding protein of chick brain (20).

The stability of the binding site was measured in protein that had been incubated for varying times at 37°C or 2°C and then allowed to bind colchicine-<sup>3</sup>H for 60 min at 37°C. As shown in Fig. 6, the loss of ability to bind colchicine was more rapid at 37°C than at 2°C. Half-lives at 2°C ranged from 4 to 9 hr, whereas those at 37°C ranged from 1.9 to 3.1 hr. It has been shown that maximal protection of the colchicine binding site of the brain protein is afforded when both  $Mg^{++}$  and  $1 \cdot 10^{-4}$  M GTP

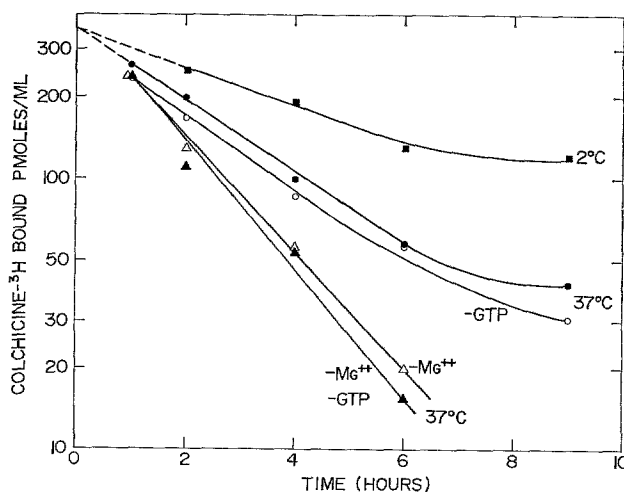


FIGURE 6 Decrease in colchicine-<sup>3</sup>H binding to soluble protein from beef thyroid as a function of time and conditions of incubation. Thyroid supernatant solutions were prepared in sucrose-phosphate, and  $1 \cdot 10^{-2}$  M of  $Mg^{++}$  and/or  $1 \cdot 10^{-4}$  M were GTP added as indicated. Samples were incubated at 2° or 37°C. Colchicine-<sup>3</sup>H binding was measured with  $5 \cdot 10^{-6}$  M colchicine for 1 hr at 37°C at the end of the preincubation period. All points are duplicate assays.

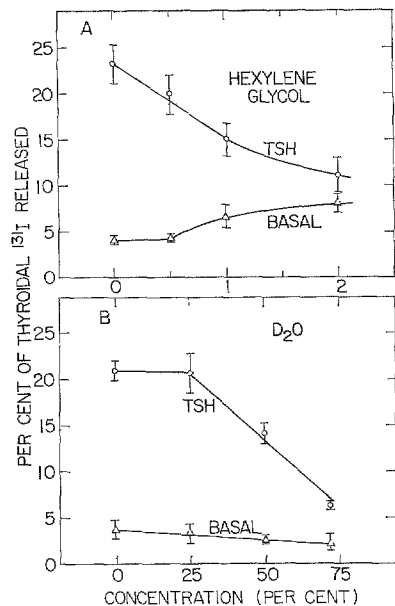


FIGURE 7 Effect of hexylene glycol (A) and D<sub>2</sub>O (B) on basal and TSH-stimulated <sup>131</sup>I release from mouse thyroids labeled for 2 hr in vivo and incubated for 4 hr in vitro. The concentration of TSH used in all cases is 2.5 munits/ml. Each point is the mean  $\pm$ SE of four or five thyroids.

are present (9, 20). As shown in Fig. 6, the major contribution to the stability of thyroid colchicine-binding activity derived from Mg<sup>++</sup> rather than GTP. Binding activity did not always decay exponentially at longer preincubation times. Extrapolation of the decay curves to zero time (20) reveals that the various deletions from the incubation medium did not significantly influence the initial binding of colchicine-<sup>3</sup>H but acted primarily on the rate of decrease of binding.

Certain other reagents have been stated to influence the stability or binding of colchicine to the binding protein (9, 20). However, with the crude thyroidal binding protein, ascorbic acid, dithiothreitol, sodium glutamate, or KCl were without effect.

#### Other Antimitotic Agents

Colchicine, which disassembles microtubules, and vinblastine, which leads to the formation of paracrystalline aggregates of microtubules, both inhibit iodine secretion from the thyroid gland (5). Another class of compounds, typified by hexylene glycol (2-methyl-2,4-pentanediol) and by D<sub>2</sub>O,

interferes with a microtubule-dependent process (mitosis) by stabilizing microtubules (21-23). If thyroid secretion were indeed dependent not only on intact microtubules but also on their ability to rearrange, etc., then hexylene glycol and D<sub>2</sub>O might be expected to inhibit thyroidal <sup>131</sup>I release. As shown in Fig. 7, this was found to be the case over the concentration range 0.5-2.0% hexylene glycol and at >25% D<sub>2</sub>O in the medium. Hexylene glycol caused a slight increase in basal release of <sup>131</sup>I, which we interpret as indicative of tissue damage (6). 70% D<sub>2</sub>O caused no such damage. No preincubation was required to achieve this inhibition with either agent. Colloid droplet accumulation was inhibited as well as <sup>131</sup>I release. Thus, colchicine, vinblastine, and D<sub>2</sub>O and hexylene glycol, which are believed to affect microtubular function by different mechanisms, all inhibit thyroid secretion.

Another approach in relating the antimicrotubular activity to binding of colchicine to protein is made possible by the fact that the antimitotic and antitumor activities of numerous colchicine analogues have been quantified (24). For this reason we have compared such analogues for their ability to influence iodine secretion from the thyroid on the one hand and binding to thyroidal protein on the other. Binding of analogues was measured by their ability to prevent binding of colchicine-<sup>3</sup>H. In Table III are listed various

TABLE III  
Comparison of the Potency of Colchicine Analogs on Inhibition of Thyroid Secretion and Binding of Colchicine-<sup>3</sup>H to Thyroid Protein

Agents	50% inhibitory* concentration on <sup>131</sup> I release	Colchicine- <sup>3</sup> H binding † (% of control)
<i>M</i>		
Colchicine	$2 \times 10^{-7}$	27.5
Colcemid	$6 \times 10^{-7}$	34.7
N-Acetylthio-colchinal	$1 \times 10^{-5}$	57.5
Colchicine	$3 \times 10^{-5}$	88.5
Colchicoside	No effect at $3 \times 10^{-4}$	96.4

\* Preincubation time of 2 hr in all experiments.

† Control binding (100%) was determined by incubation of beef thyroid SMPG supernatant solution with  $1 \times 10^{-6}$  M, colchicine-<sup>3</sup>H for 1.5 hr at 37°C. The various analogues were added simultaneously at  $1 \times 10^{-5}$  M.

analogues arranged in the order of decreasing antimitotic activity (24). There is a striking correlation of the antisecretory potency with the ability of these analogues to interact with the colchicine-binding protein. These data thus provide strong evidence that the antisecretory effects of colchicine are operating through an interaction with the microtubular protein.

#### DISCUSSION

Three major questions arise which can be answered with a decreasing level of certainty: (a) Is the inhibitory action of the microtubule-active agents mediated through an action on the microtubule? (b) Is the inhibitory effect on secretion specific to microtubules or colchicine-binding protein? (c) What is the role of microtubules in thyroid secretion at the microscopic or molecular level?

A *sine qua non* for the present discussion is the need to establish that the colchicine-binding protein is, in fact, a component subunit of microtubules. This has been widely assumed on the basis of colchicine effects on the state of aggregation of mitotic spindles and certain other microtubular structures. Actually, most of the evidence is indirect. Thus, colchicine binding is high in tissues that have abundant microtubules and low in those that do not (25), and isolated mitotic apparatus preparations are enriched in colchicine-binding protein (15). Moreover, specific removal of the central pair of microtubules from sea urchin sperm tails progressively removes the colchicine-binding activity from the sperm tails and is accompanied by a corresponding increase of such activity in the extract (26). More direct evidence derives from experiments of Kirkpatrick et al. (22) who showed that the major protein obtained from isolated brain microtubules characterized on the basis of ultrastructure behaves electrophoretically like the colchicine-binding protein isolated directly from brain and has the same molecular weight. Furthermore, antibodies made to preparations from *Tetrahymena* cilia of the type that yield colchicine-binding protein appear to combine with intact bundles of microtubules (27). Using another approach, it has been shown that vinblastine-induced paracrystalline precipitates of microtubules (28–30) show *in vivo* radioautographic localization of colchicine-<sup>3</sup>H (31). The isolated paracrystals also have colchicine-binding activity *in vitro* and have an amino acid composition similar to that reported for colchicine-binding protein and other presumptive microtubular subunits (32).

The thyroid cell contains numerous, apparently unorganized, cytoplasmic microtubules (33). It also contains a colchicine-binding protein which resembles that of brain and other tissues on the basis of size (6S) and behavior on Sephadex (5), the apparent  $K_m$  (Fig. 5), the temperature and pH dependence, and the lability (Fig. 5) of colchicine binding. These similarities, taken together with the excellent correlation of the antimitotic potency of a number of colchicine analogues with their effect on both secretion and colchicine binding (Table III), provide strong support for the presumption that colchicine affects microtubular stability in the thyroid in a manner similar to that of other tissues.

The likelihood that the effect of colchicine on secretion operates through microtubules in no way rules out the possibility that there might be other actions of colchicine that could influence the secretory process. In examining this question, it is important to distinguish between those metabolic processes commonly, or obligatorily, associated with secretion, which might be influenced by interference with microtubule function, from other effects. To this end we have recently shown that colchicine concentrations that completely inhibit iodine secretion or colloid droplet accumulation do not inhibit the TSH-stimulated oxidation of [<sup>1-<sup>14</sup>C</sup>]glucose, [<sup>6-<sup>14</sup>C</sup>]glucose, or [<sup>1-<sup>14</sup>C</sup>]pyruvate (34). Very large concentrations of colchicine ( $1 \cdot 10^{-2}$  M) may inhibit glucose oxidation (10), but such levels are  $\sim 10^4$  times those required to inhibit secretion and they have not been used in the present study.

We have shown earlier that colchicine does not inhibit fluoride- or TSH-stimulated beef thyroid adenylate cyclase or the cyclic 3',5'-nucleotide phosphodiesterase of mouse thyroid glands (5). The present results, showing no effect on colchicine on the tissue levels of cyclic AMP (Table II), are in accord with the above conclusions. We have also been able to show that colchicine has no effect on iodide transport by the mouse thyroid, nor on the *in vitro* uptake or short term conversion of <sup>131</sup>I<sup>-</sup> to iodoamino acids (unpublished observations). It has also been shown that hormone induction of mammary tissue dehydrogenases is not affected by colchicine (35).

Since membrane-stabilizing drugs may have marked effects on secretory processes including those in the thyroid, it is possible that colchicine and related drugs might act as membrane stabilizers. This would appear to be unlikely, how-



ever, on the basis of the great water solubility of colchicine and the fact that no membrane stabilization has been found in the lysosomal membrane (36) or the erythrocyte (K. Matthews, personal communication). The absence of a colchicine effect on adenylate cyclase, which is very sensitive to membrane stabilizers (37), also argues against such an effect.

It may be concluded, then, that after stimulation of the TSH receptor the early formation of colloid droplets and their intracellular accumulation is highly sensitive to inhibition by colchicine. The participation of microtubules in this process may be considered as highly probable, although the nature of the microtubular contribution to hormone secretion may be either active or passive (structural).

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