

## Reaccumulation of thyroglobulin and colloid in rat and mouse thyroid follicles during intense thyrotropin stimulation. A clue to the pathogenesis of colloid goiters.

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### Research Article

Since Marine's observations some 50 years ago, it has been generally accepted that colloid goiters invariably result from colloid depletion of originally hyperplastic goiters after cessation of the goitrogenic stimulus. However, clinical observations suggest that many goiters never go through a stage of hyperplasia, but are colloid-rich from the beginning. We have injected rats and mice with thyrotropin (TSH), three times a day for 4 d, while the animals were kept on an iodine-rich diet (HID). Additional groups of animals were fed an iodine-poor diet (LID) or a diet containing 0.15% propylthiouracil (PTU) or 1% sodium perchlorate (ClO<sub>4</sub>). At intervals, thyroid weight, DNA, iodine and thyroglobulin content, thyroglobulin iodination, and intracellular droplet formation were measured. Histologic sections were also prepared and stained with periodic acid Schiff. Furthermore, thyroxine concentration was measured in the serum. Thyroglobulin content dropped by approximately 30% in HID animals but by 60% in all other groups 1 d after starting TSH. Thereafter, thyroglobulin reaccumulation occurred and droplet formation correspondingly decreased despite continuous heavy TSH stimulation. The largest amount of thyroglobulin was reaccumulated in HID animals followed by the PTU/LID groups, whereas no reaccumulation was observed in the ClO<sub>4</sub> group. Reaccumulation of thyroglobulin only occurred if there was concomitant organification of at least some iodine. The subsequent phases of depletion and reaccumulation of thyroglobulin were mirrored by [...]

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# Reaccumulation of Thyroglobulin and Colloid in Rat and Mouse Thyroid Follicles during Intense Thyrotropin Stimulation

## A CLUE TO THE PATHOGENESIS OF COLLOID GOITERS

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**ABSTRACT** Since Marine's observations some 50 years ago, it has been generally accepted that colloid goiters invariably result from colloid repletion of originally hyperplastic goiters after cessation of the goitrogenic stimulus. However, clinical observations suggest that many goiters never go through a stage of hyperplasia, but are colloid-rich from the beginning.

We have injected rats and mice with thyrotropin (TSH), three times a day for 4 d, while the animals were kept on an iodine-rich diet (HID). Additional groups of animals were fed an iodine-poor diet (LID) or a diet containing 0.15% propylthiouracil (PTU) or 1% sodium perchlorate (ClO<sub>4</sub>). At intervals, thyroid weight, DNA, iodine and thyroglobulin content, thyroglobulin iodination, and intracellular droplet formation were measured. Histologic sections were also prepared and stained with periodic acid Schiff. Furthermore, thyroxine concentration was measured in the serum.

Thyroglobulin content dropped by ~30% in HID animals but by 60% in all other groups 1 d after starting TSH. Thereafter, thyroglobulin reaccumulation occurred and droplet formation correspondingly decreased despite continuous heavy TSH stimulation. The largest amount of thyroglobulin was reaccumulated in HID animals followed by the PTU/LID groups, whereas no reaccumulation was observed in the ClO<sub>4</sub> group. Reaccumulation of thyroglobulin only occurred if there was concomitant organification of at least some iodine. The subsequent phases of depletion and re-

accumulation of thyroglobulin were mirrored by the morphology of the follicular lumina, the staining properties of the colloid and the serum T<sub>4</sub> concentration. These observations suggest that endocytosis gradually becomes refractory to continuous TSH stimulation if a certain minimal amount of iodine is available for organic binding. Thus, primarily colloid-rich goiters may form in the presence of continuously higher than normal thyrotropin levels without a previous stage of follicular hyperplasia. The view should be revised that accumulation of colloid and intense thyrotropin stimulation are mutually exclusive events.

## INTRODUCTION

Experimental stimulation of the thyroid gland by thyrotropin (TSH)<sup>1</sup> causes the growth of hyperplastic goiters characterized by colloid-depleted follicles (1). Colloid endocytosis is an almost immediate response of the follicular cell to TSH (1, 2). On the other hand, human euthyroid goiters are generally considered to be the consequence of chronic excessive TSH secretion although they are usually not of the hyperplastic type, but contain a widely varying number of large, colloid-filled follicles (3-6). The apparent paradox of simultaneous TSH stimulation and excessive colloid storage has not been adequately explained.

Marine's early hypothesis (7) postulating that colloid goiters result from transformation of originally hyperplastic glands after cessation of the goitrogenic stimulus, has never been really challenged. Rather, it has found apparent experimental support, after the pituitary-

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<sup>1</sup>Abbreviations used in this paper: HID, high iodine diet; LID, low iodine diet; PAS, periodic acid Schiff; PTU, 6-n-propylthiouracil; TSH, thyrotropin.

thyroid feedback control had become known, by data demonstrating that colloid reaccumulation occurs as a consequence of intrinsic autonomous properties of any TSH-stimulated follicle as soon as pituitary secretion of this hormone is suppressed (8–10). However, little attention was given to the fact that true colloid goiters with higher than normal thyroglobulin content have never been achieved by any experimental procedure, nor was it realized that the coexistence of hyperplastic, highly cellular areas with colloid-rich hypocellular regions in most simple goiters is hardly compatible with Marine's hypothesis (3). Therefore, the possibility should be reinvestigated that colloid accumulation could, in certain circumstances, occur in the presence of supra-normal TSH stimulation. The present paper demonstrates that in rats and mice intra-follicular thyroglobulin reaccumulation can indeed occur despite intense continuous TSH stimulation and that availability of a certain minimal iodine supply is a requisite to this phenomenon. In the context of this finding a number of observations are reviewed that suggest that a similar mechanism may explain many facets of human colloid goiter.

## METHODS

400 male Wistar rats weighing 250–350 g and 55 male mice (inbred from the ICR strain of the Institute of Zuchtthygiene, University of Zürich) were used. All animals were bred in our institute. Up to the beginning of and, if not stated otherwise, during the experiment the animals were kept on an iodine-rich stock diet (HID), containing  $\sim 1.5 \mu\text{g}$  iodine/g. Some animals were fed a moderately low iodine diet and some a high iodine diet containing 0.15% 6-*n*-propylthiouracil (PTU). This dose of PTU effectively, but never completely (11), blocks organic binding of iodine in resting mice thyroids, since only  $0.021 \pm 0.008\%$  (SEM) ( $n = 4$ ) of an  $^{125}\text{I}$  dose are organified within 4 h. However, with intense TSH stimulation organic binding always increases to a considerable extent, e.g. to  $0.392 \pm 0.19\%$ , if PTU is given together with HID. The data are in accord with data published by Astwood and Bissell (12). The diets were obtained from Altromin (Lage, Germany). All animals had free access to tap water. For two experimental groups, 1% NaClO<sub>4</sub> was added to the drinking water. For some animals (mice and rats) T3 (Sigma Chemical Co., St. Louis, Mo.) was added to the drinking water (0.2  $\mu\text{g}$  T3/ml). After treatment with bovine TSH (Ambinon, Organon) up to 4 d all the animals were killed by exsanguination under diethyl ether anesthesia, 2 h after the last TSH injection.

For the isolation of thyroglobulin two or three thyroids were homogenized with an all glass homogenizer in 0.5 ml sodium phosphate buffer (0.1 M) and centrifuged for 1 h at 100,000 *g*. The pellet was gently rehomogenized in 0.4 ml buffer and recentrifuged. The two supernatants were pooled and applied on an 8-ml linear gradient of 10–40% sucrose in the same buffer and spun at 4°C for 17 h at 37,000 rpm (IEC, B-60 ultracentrifuge, SB-283 rotor IEC, Div. of Damon Corp., Needham Heights, Mass.).

Fractions of four drops were collected and the optical density at 280 nm was measured. The fractions containing the 19S thyroglobulin peak were pooled. The protein concentration of the pool was calculated by measuring the absorbance at 280 nm ( $E_{1\%}^{1\text{cm}} = 10$ ).

Iodine in the homogenate and in the thyroglobulin pool was measured by a kinetic method using the catalytic activity of iodide (13). The thyroids were fixed in Bouin's fluid. After routine histological preparation sections of 6  $\mu\text{m}$  were stained with periodic acid Schiff (PAS). Intracellular colloid droplets were counted in some of the sections according to Shishiba et al. (14).

DNA was determined in duplicate in 0.2 ml of the homogenate with the diphenylamine reaction according to the method described by Burton (15), with a minor modification: the color was developed by incubating at 4°C for 16 h.

Serum T4 was measured with a commercial radio-immunoassay (Tetra-Tab-RIA, NML).

Student's *t* test was used for the statistical analysis of the data.

## Experiment I

Rats in four experimental groups (30 animals per group) were fed HID and injected with TSH intraperitoneally as follows:

*Group I.* Control: saline.

*Group II.* 9 h TSH: 0.5 IU TSH at 8 a.m., 11:30 a.m. and 3 p.m., killed at 5 p.m., i.e. 9 h after the first TSH injection.

*Group III.* 2 d TSH: 0.5 IU at 8 a.m. and noon, 2 IU at 5 p.m. for 2 d, 0.5 IU TSH at 8 a.m. of day 3; killed at 10 a.m. of that day.

*Group IV.* 4 d TSH: treatment as for group III, but for 4 d. At day 5 injected at 8 a.m. with 0.5 IU TSH and killed at 10 a.m.

The thyroids were homogenized (three glands pooled) and total thyroidal iodine (measured in the homogenate), thyroidal thyroglobulin content and thyroglobulin iodination were determined.

## Experiment II

Rats were injected with 1 IU TSH at 8-h intervals (8 a.m., 4 p.m., and midnight) up to 4 d. Untreated controls and four groups of TSH-injected animals (10 h TSH, 1 d TSH, 2 d TSH, and 4 d TSH) were fed the HID. Six additional groups were treated with TSH for 1 or 4 d and kept on either LID, PTU, or HID + ClO<sub>4</sub>. 12–38 animals were used per group. For some animals T3 was added to the drinking water (0.2  $\mu\text{g}$  T3/ml) to suppress any possible endogenous TSH secretion.

The thyroids were used for histologic preparation and droplet counting or they were weighed and homogenized for determination of thyroidal thyroglobulin and thyroglobulin iodination. Serum of some animals was frozen for T4 measurement.

The experiment was repeated and in addition to the parameters just mentioned, thyroidal DNA content was measured.

## Experiment III

Mice kept on HID were injected with TSH (200 mU) and killed after 2 or 8 h (group II and III). Mice kept on HID, LID, PTU, or HID + ClO<sub>4</sub> were injected with TSH (200 mU) twice a day up to 4 d (group IV–X). Groups VIII–X were given 0.2  $\mu\text{g}$  T3 per ml in the drinking water in order to suppress any possible endogenous TSH secretion. The 10 experimental groups (five to six mice each) were characterized as follows:

I	control (HID)	VI	4 d TSH (HID)
II	2 h TSH (HID)	VII	4 d TSH (LID)
III	8 h TSH (HID)	VIII	4 d TSH (HID, T3)
IV	1 d TSH (HID)	IX	4 d TSH (ClO <sub>4</sub> , T3)
V	2 d TSH (HID)	X	4 d TSH (PTU, T3)

All thyroids were used for histology only.

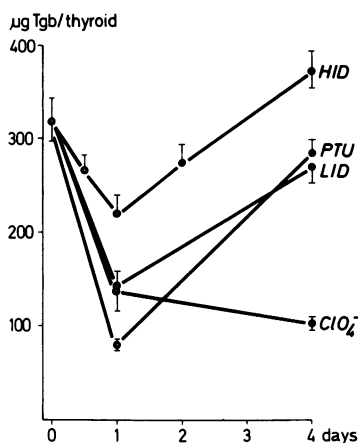


FIGURE 1 Thyrogllobulin (Tgb) content of rats injected with 1 IU TSH three times per day and fed a normal (HID), a LID, and a PTU-containing diet. The animals of one group were given perchlorate (CIO<sub>4</sub>) in the drinking water. Means  $\pm$  SE of 5 to 15 values, i.e. 15 to 45 glands, are given.

1 d TSH HID vs. control, vs. 1 d TSH LID and 1 d TSH CIO<sub>4</sub>,  $P < 0.02$   
 1 d TSH PTU/LID/CIO<sub>4</sub> vs. control,  $P < 0.001$   
 1 d TSH PTU vs. 1 d TSH LID,  $P < 0.01$ , vs. 1 d TSH CIO<sub>4</sub>,  $P < 0.05$  and vs. 1 d TSH HID,  $P < 0.0001$   
 4 d TSH HID vs. control ns, vs. 1 d TSH HID,  $P < 0.001$  and vs. 2 d TSH HID,  $P < 0.01$   
 4 d TSH PTU vs. 1 d TSH PTU,  $P < 0.001$  and vs. 4 d TSH HID,  $P < 0.02$   
 4 d TSH LID vs. 1 d TSH LID and 4 d TSH HID,  $P < 0.001$   
 4 d TSH CIO<sub>4</sub> vs. 1 d TSH CIO<sub>4</sub> ns and vs. 4 d TSH HID/LID/PTU,  $P < 0.001$

## RESULTS

Thyroidal thyrogllobulin content for rats on various diets and injected up to 4 d with TSH (experiment II) is shown in Fig. 1. During the first day of TSH stimulation a rapid loss of thyrogllobulin occurred. Thyrogllobulin depletion was much more marked in animals on LID, PTU, and CIO<sub>4</sub> than in those on ample iodine supply, suggesting that impaired iodination increases the endocytotic response to TSH. If TSH stimulation continued, the thyroids of animals on HID as well as those on LID and PTU were repleted, but not those on CIO<sub>4</sub>. The net balance is given in Fig. 1. As expected from previous experiments and confirmed by the levels of thyrogllobulin iodination (Table I), neither PTU nor moderately severe LID can prevent significant iodine reaccumulation if the thyroid glands are severely stimulated by TSH, whereas CIO<sub>4</sub> is obviously more effective. Therefore, thyrogllobulin reaccumulation goes along with, and might possibly require, accumulation of organic iodine in the thyroid. The sensitizing effect of CIO<sub>4</sub> on iodine release (16) and droplet formation (Fig. 3 and Table II) might be related to its impact on iodine accumulation.

Total thyroidal iodine content increased considerably during TSH treatment if enough iodine was available in the diet. Therefore, in experiment I, total thyroidal iodine (microgram per gland) rose from  $6.68 \pm 0.47$  (mean  $\pm$  SE,  $n = 10$ ) to  $8.97$  after 2 d TSH and to  $9.79 \pm 0.75$  after 4 d TSH (both  $P < 0.01$ ). Goiters resulting from continuous TSH stimulation were iodine-rich, if

TABLE I  
Thyrogllobulin Iodination during "Continuous" TSH Stimulation\*

	Control	½ d TSH	1 d TSH	2 d TSH	4 d TSH
HID	$0.78 \pm 0.04 \ddagger$ (11)	$0.58 \pm 0.03 \S$ (5)	$0.66 \pm 0.08^{\parallel}$ (5)	$0.80 \pm 0.05$ (5)	$0.85 \pm 0.03 \S \S$ (15)
LID			$0.63 \pm 0.02^{\parallel \parallel}$ (6)		$0.46 \pm 0.02^{\parallel \parallel \parallel}$ (16)
CIO <sub>4</sub>			$0.54 \pm 0.03^{**}$ (6)		$0.21 \pm 0.02^{\parallel \parallel \parallel}$ (5)
PTU			$0.51 \pm 0.02 \ddagger \ddagger$ (6)		$0.55 \pm 0.03$ (5)

\* Experiment II. Micrograms iodine/100 µg thyrogllobulin.

‡ Mean  $\pm$  SEM, in brackets n.

§  $P < 0.01$  vs. control.

|| NS vs. control.

|||  $P < 0.02$  vs. control.

\*\*  $P < 0.02$  vs. control and ns vs. 1 d TSH HID.

‡‡  $P < 0.001$  vs. control and ns vs. 1 d TSH HID.

§§  $P < 0.02$  vs. 1 d TSH HID.

|||  $P < 0.001$  vs. 1 d TSH LID, 0.0001 vs. 4 d TSH HID and  $P < 0.05$  vs. 1 d TSH PTU.

|||  $P < 0.0001$  vs. all other values.

TABLE II  
*Intracellular Colloid Droplets 2 h after 1 IU TSH in "Continuously" Stimulated Rat Thyroids*

	Before TSH	2 h TSH	1 d TSH	4 d TSH	4 d TSH + ClO <sub>4</sub> 2 h before death
Animals per group	5	5	6	6	3
Droplets per nucleus	0.06±0.01*	3.74±0.16	1.94±0.21‡	1.02±0.14§	3.39±0.17 <sup>  </sup>
Droplets per 25 follicles	50±11*	2,600±143	1,269±158‡	653±90§	2,688±188 <sup>  </sup>

Experiment II. High iodine diet. All animals were killed 2 h after the last TSH injection.

\*  $P < 0.0001$  vs. all other values.

‡  $P < 0.001$  vs. 2 h TSH.

§  $P < 0.0001$  vs. 2 h TSH and  $P < 0.01$  vs. 1 d TSH.

<sup>||</sup>  $P < 0.0001$  vs. 4 d TSH.

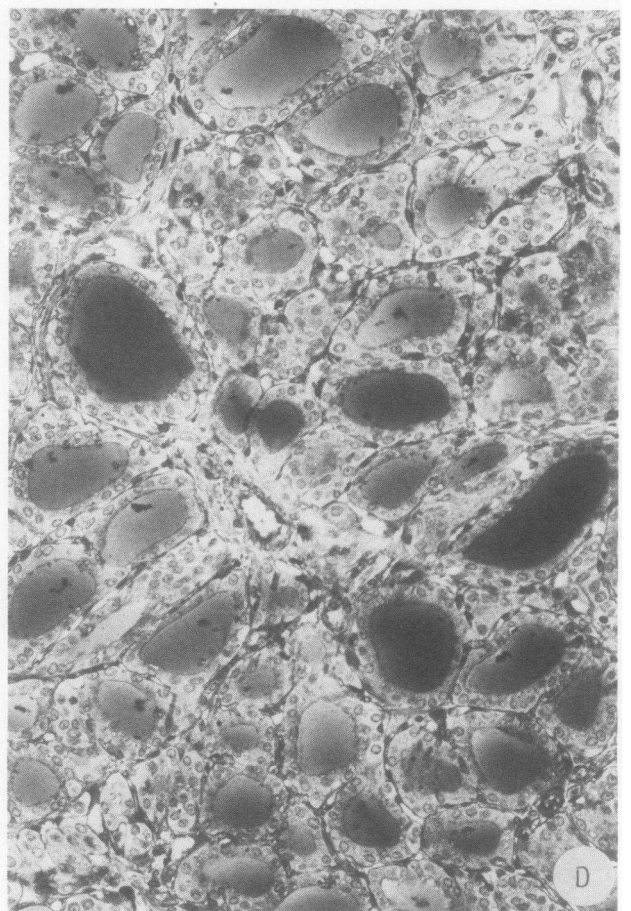
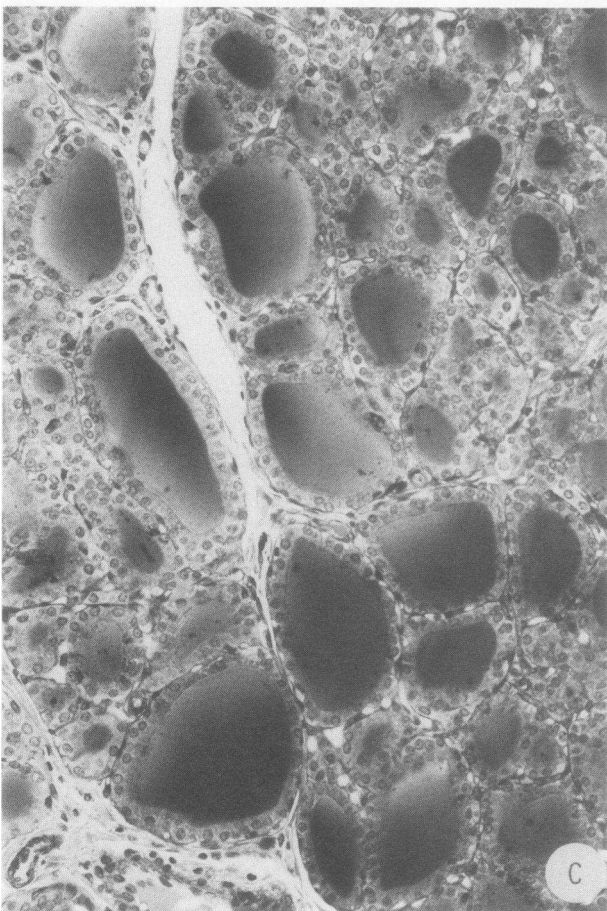
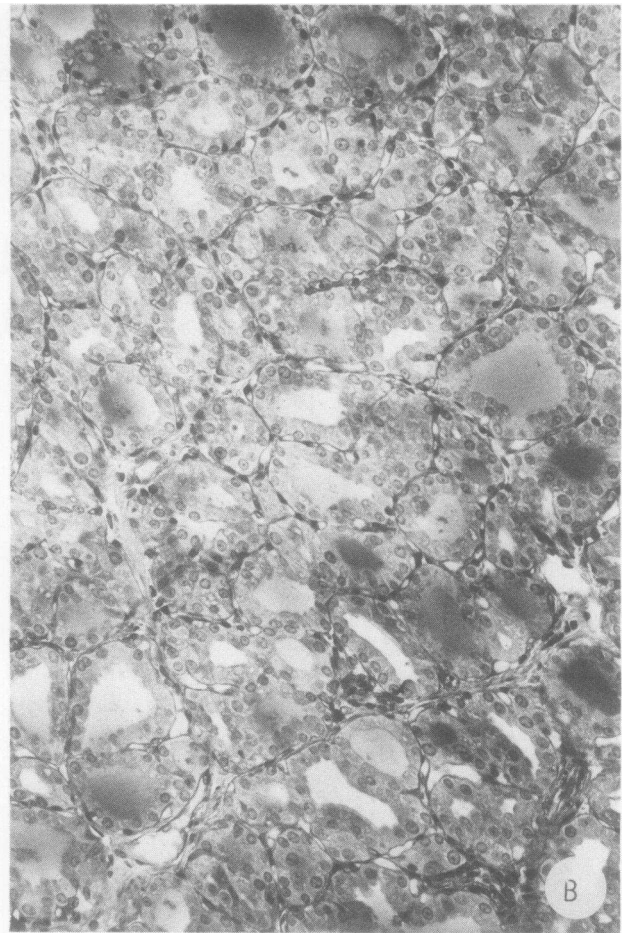
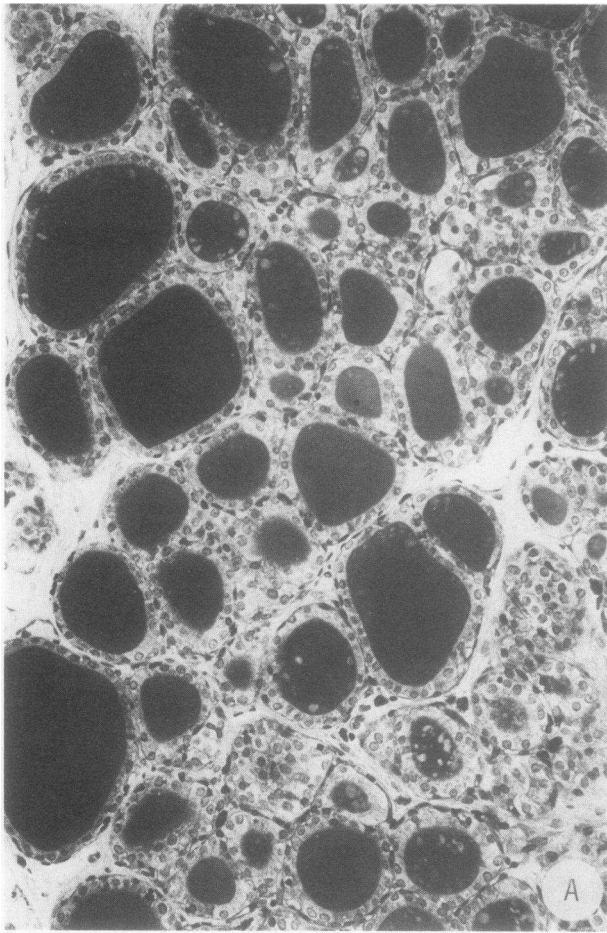
the animals were fed a HID. Table I summarizes the effect of TSH treatment and the different diets on thyroglobulin iodination. Under HID, when much iodine was available, thyroglobulin iodination slightly dropped initially, but returned to control levels during TSH stimulation. Constant thyroglobulin iodination together with reaccumulation of lost thyroglobulin are consistent with the increasing thyroidal iodine content found with prolonged TSH stimulation. Part of this iodine is transferred to slow-turnover compartments (6, 17, 18). After 1 d of TSH treatment the thyroglobulin iodine content of iodine-deficient animals was not different from the values observed in animals on the iodine-rich diets, whereas ClO<sub>4</sub> or PTU treatment resulted in a slightly lower iodine content (Table I). After 4 d of TSH stimulation iodine-deficient and PTU-treated animals had a lower thyroglobulin iodination than animals on ample iodine supply. PTU cannot completely block iodination when given together with large doses of TSH (see above). Perchlorate treatment is apparently more efficient in preventing TSH-stimulated iodine accumulation, thus causing the lowest degree of thyroglobulin iodination.

The course of thyroglobulin depletion and repletion shown in Fig. 1 is confirmed by the histologic appearance of the follicles, as illustrated in Fig. 2. After 1 d of TSH treatment most follicles were stimulated, showing smaller lumina and containing less, or less concentrated, PAS-positive material such as thyroglobulin (Fig. 2B). Within 4 d large amounts of colloid reappeared in the follicular lumina (Fig. 2C). The colloid was still somewhat less PAS-stained than in controls, but otherwise the heavily stimulated glands were similar to untreated controls except for the presence of a slightly higher number of follicles that had some evidence of stimulation, such as scattered droplet formation and a somewhat higher epithelium. In LID- and PTU-treated rats, these signs of stimulation were clearly more marked than in HID controls, whereas in perchlorate-treated animals most follicles remained fully stimulated, containing little colloid.

Identical results were obtained in rats and mice, namely depletion and full repletion under TSH + HID, less complete repletion under TSH + LID and TSH + PTU, and no repletion under TSH + ClO<sub>4</sub>. Because the difference between HID, LID, and PTU on one hand and ClO<sub>4</sub> on the other invariably existed whether or not the mice were simultaneously treated with T<sub>3</sub>, endogenous TSH secretion can be ruled out as a possible factor intervening in producing the observed results.

Droplets were counted in thyroids of most experimental groups. The droplet number 2 h after the last TSH injection and therefore the pinocytotic response to TSH decreased rapidly within 1 d after the onset of TSH treatment and even more so after 4 d. If a single dose of 50 mg NaClO<sub>4</sub> was injected together with the last TSH dose, droplet formation was immediately restored (Fig. 3D). In Table II the number of droplets is given. It should however be stressed that quantitative comparison is only permitted between follicles with similar colloid content such as the HID rats. Severely hyperplastic glands such as those of ClO<sub>4</sub> animals, cannot form many droplets. For these reasons, droplet counts in LID and PTU rats, which were in fact identical to those of HID animals at 4 d TSH, are not numerically indicated in Table II.

Fig. 4 gives the serum T<sub>4</sub> values measured in TSH stimulated rats. To understand these data one must recall the response of serum hormones in rats treated with PTU and ClO<sub>4</sub> but not with TSH (19, 20): characteristically, there is a quick and marked drop. Thus, the actual experimental values indicate important TSH-induced T<sub>4</sub> release on day 1 even in PTU and ClO<sub>4</sub> rats. Because of increasing resistance of secretory mechanisms with ongoing TSH stimulation, the serum T<sub>4</sub> fails to increase further even in HID animals and also in LID animals where some iodine is still available: it drops to near pretreatment levels. The maintenance of nearly normal serum T<sub>4</sub> concentration in PTU animals is in line with a less resistant secretory machinery and an incomplete block of iodination. The





sharp decline of T4 in ClO4 rats mirrors the severe exhaustion of hormone stores between days 1 and 4 as indicated in Figs. 1 and 3. We have indeed found in other experiments (21) that the fraction of thyroglobulin iodine contained as thyroid hormones remains constant in this type of experiment. Therefore the total thyroidal hormone content is proportional to the total thyroglobulin iodine.

Thyroid weight and total thyroid DNA content increased consistently on the fourth, but not on the first day of TSH treatment with no statistically significant differences between the groups with the four different treatments. A typical experiment yielded a control weight of  $12.2 \pm 0.6$  mg ( $n = 10$ ,  $\pm$ SEM) with weights of  $17.4 \pm 0.9$  (HID),  $17.8 \pm 1.0$  (LID),  $18.8 \pm 0.6$  (PTU) and  $16.3 \pm 1.1$  (ClO4) after 4 d of TSH. Total thyroidal DNA (micrograms per gland) also rose within 4, but not after 1 d TSH, from a control value of  $52.0 \pm 4.4$  ( $n = 5$ ,  $\pm$ SEM) to  $67.0 \pm 7.2$  (HID),  $69.6 \pm 5.9$  (LID),  $60.3 \pm 4.6$  (PTU) and  $67.4 \pm 4.9$  (ClO4). Only the PTU group falls short of statistical significance ( $P < 0.05$ ).

Immunological inactivation of heterologous TSH within 4 d of treatment was excluded as follows: (a) There was thyroid hyperplasia and severe hyperemia after 4 d of treatment. (b) Total DNA was increased after 4 d but not after 1 d TSH. (c) Animals that were treated simultaneously with ClO4, T3, and TSH did not show any reaccumulation of thyroglobulin nor morphological refilling of follicles. (d) Persisting TSH effectiveness was definitely proved by giving a single dose of 50 mg NaClO4 i.p. together with the last TSH injection to three rats that had refilled their follicular lumina after 4 d TSH + HID treatment. Intense droplet formation was restored (Fig. 3, Table II).

## DISCUSSION

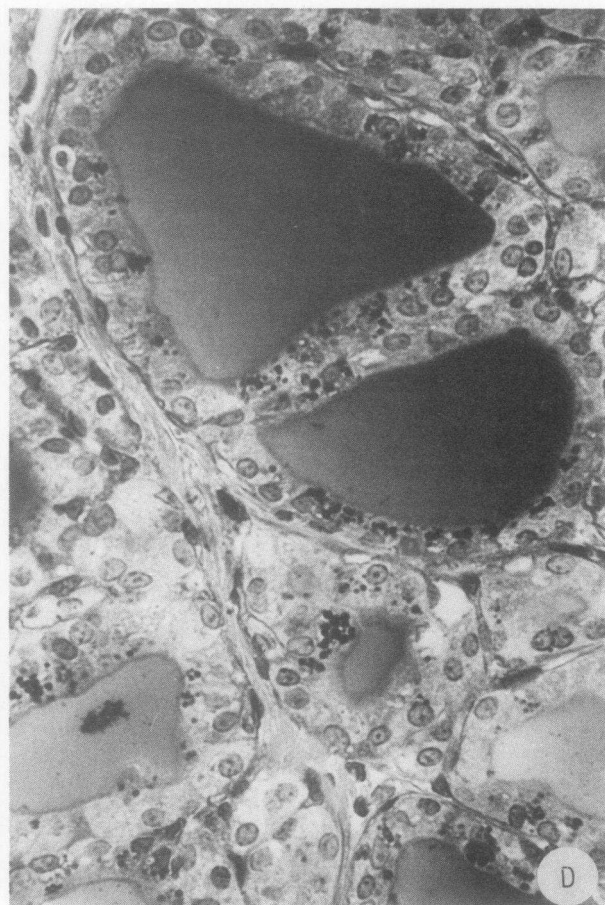
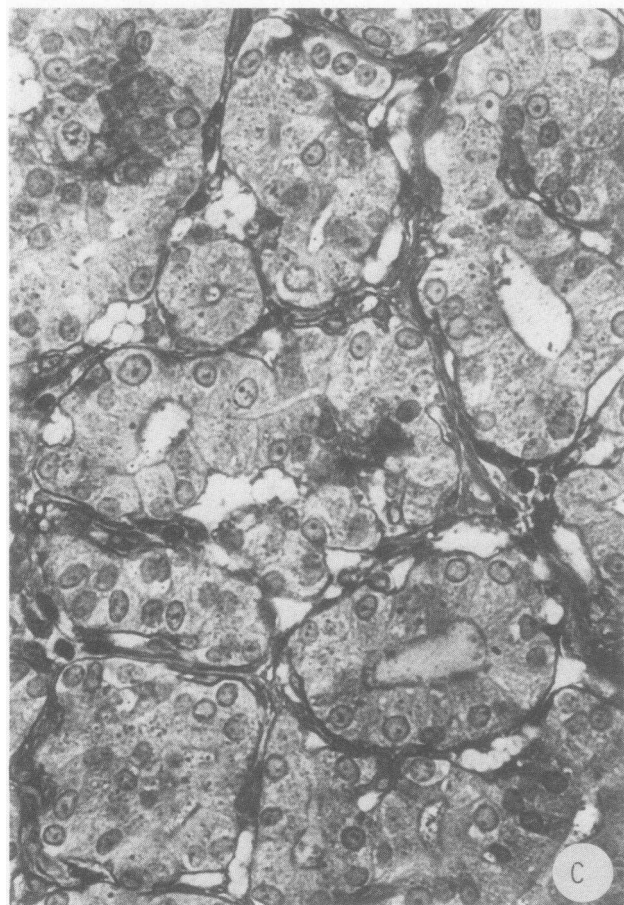
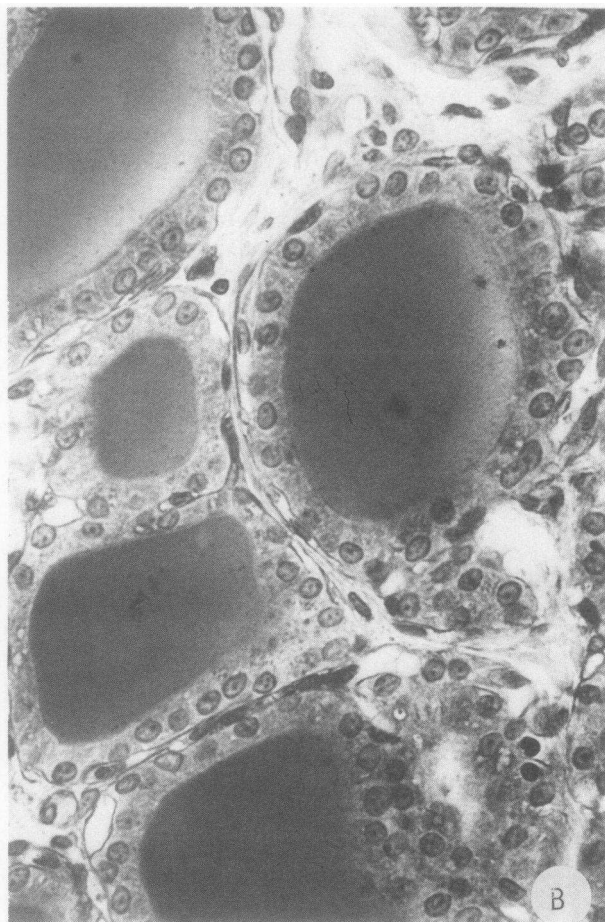
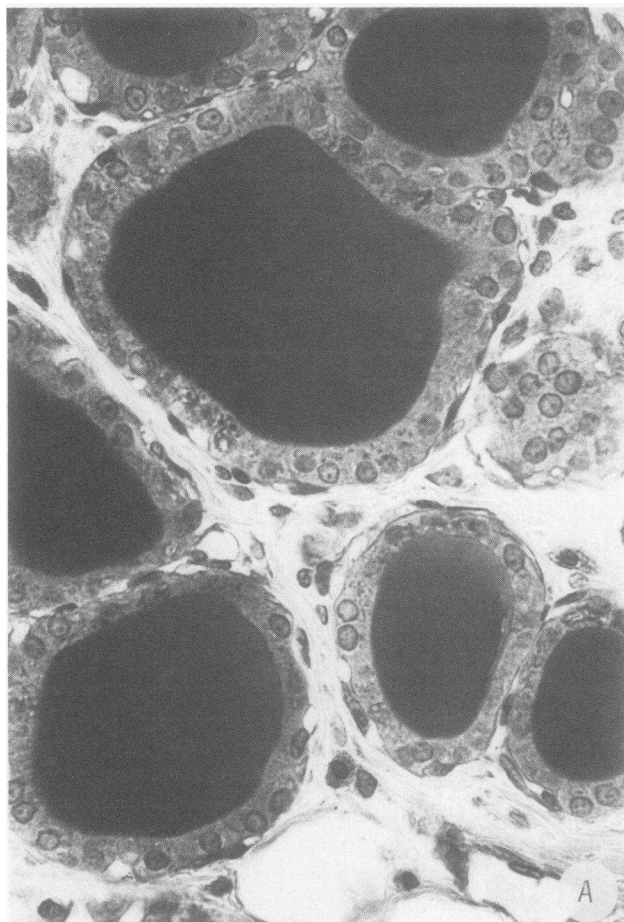
The main points demonstrated in the present work are (a) intense TSH stimulation of thyroid follicles and simultaneous colloid accumulation within the follicular lumina are not mutually exclusive events and (b) TSH only has a full stimulating effect on endocytosis if simultaneous organic binding of iodine is severely depressed.

In rats and mice on ample iodine supply, a single injection of TSH causes the prompt appearance of numerous intracellular droplets and the loss of 30% of stored thyroglobulin within 1 d. However, if normal iodination is acutely impeded by LID, PTU, or ClO4 at the time of TSH injection, the endocytotic response

to TSH is much more effective, leading to a loss of ~60% of stored thyroglobulin. The subsequent response to further TSH injections is entirely different from that of the first dose. If we consider only HID animals, the initially shrunken follicular lumina refill with thyroglobulin (Fig. 1) and colloid (Figs. 2 and 3) in such a way that the continuously stimulated glands become morphologically similar to normal glands at the end of 4 d of TSH treatment. The severely reduced droplet formation in the presence of large colloid spaces suggest that endocytosis has become less responsive to TSH. Therefore, the normal sequence of responses of the thyroid to TSH, which invariably results in colloid depletion, is now reversed and colloid as well as thyroglobulin repletion occurs instead. The new sequence of events is also mirrored by the return to near normal levels of the initially elevated serum T4 (Fig. 4, HID rats).

Inevitably, the question must now arise why colloid reaccumulation, despite continuous TSH action, is not usually observed in goiters produced experimentally with low iodine diet and goitrogenic drugs. We first considered the possibility that the amount of iodine available for organic binding could be a key factor. Indeed, experimental goitrogenic regimens are commonly so severe that only little iodine is left to the gland, whereas human endemic goiters, which are mostly colloid rich, usually result from more chronic but less severe and more fluctuating iodine deficiency (6). We therefore injected group IV rats (4 d TSH stimulated, HID fed) with one single dose of NaClO4 together with the last TSH: The partial resistance of endocytosis was immediately abolished and normal droplet formation was restored (Fig. 3). These results strongly support the view that TSH resistance of endocytosis only occurs if there is simultaneous organification of newly entering iodide. The results obtained in LID, PTU and ClO4 treated animals were now considered with this hypothesis in mind. If animals were given a low iodine diet or a goitrogenic drug, twice as much thyroglobulin was secreted from the thyroid when compared with animals on HID (Fig. 1). This phenomenon also suggests that endocytosis is more sensitive to TSH in thyroids with acutely impaired iodination. Moreover, reaccumulation of thyroglobulin (Fig. 1) and colloid (Figs. 1–3), indicating diminishing efficiency of endocytosis, occurred with continuing TSH stimulation only in those thyroids that also organified some iodine. This occurred in PTU- and LID-treated animals, but much less so in animals

**FIGURE 2** Thyroid depletion and repletion in "continuously" TSH treated rats. Thyroid sections of a control rat (A) and of rats injected with 1 IU TSH at 8-h intervals over 1 (B) and 4 (C, D) d. A, B, and C were fed a HID, D was fed a LID. The follicular lumina were on the average somewhat smaller in D than in C, in line with a lower thyroglobulin content of LID fed animals. Note also the higher content of PAS positive material (a.o. thyroglobulin) in A than in B, C, and D.  $\times 179$ . PAS staining.





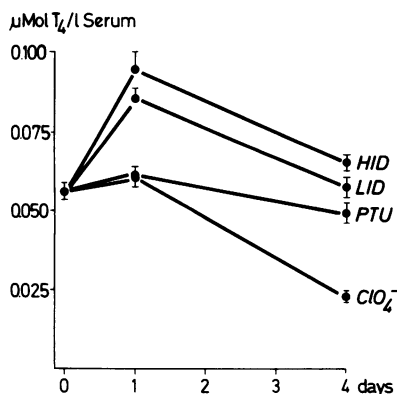


FIGURE 4 Serum T4 content of rats injected with 1 IU TSH three times daily and fed a normal (HID), a LID, or a PTU-containing diet. The animals of one group were given 1% perchlorate (ClO<sub>4</sub>) in the drinking water. Mean  $\pm$  SE of 10 to 21 values per group are given.

1 d TSH HID/LID vs. control,  $P < 0.0001$

4 d TSH HID vs. control,  $P < 0.05$  and vs. 1 d TSH HID,  $P < 0.0001$

4 d TSH ClO<sub>4</sub> vs. control and 1 d TSH ClO<sub>4</sub>,  $P < 0.0001$

4 d TSH LID vs. 1 d TSH LID,  $P < 0.0001$

4 d TSH PTU vs. 1 d TSH PTU,  $P < 0.01$

receiving ClO<sub>4</sub>. Indeed, while PTU and LID may lower, but do not block iodination in normal animals, their effect is partly overridden by the intense TSH stimulation used in the present experiments (Methods). The respective effectiveness of the four regimens is demonstrated by the different levels of iodination of the thyroglobulin present in the goiters (Table I). Therefore, reaccumulation of colloid occurs only if at least some critical amount of iodine is simultaneously organified. If not, the effect of endocytosis outweighs, at all times, that of colloid secretion and the resulting goiters remain hyperplastic.

It is yet unknown whether the inhibiting effect of organic iodine formation on endocytosis occurs through a change in the biophysical properties of the colloid or by an effect at the cellular level. While the former possibility has not yet been considered in the literature, there is growing evidence that the response of thyroidal adenyl cyclase to TSH both in vitro (22, 23) and in vivo (24, 25) is inversely related to organic binding of iodine and that iodine withdrawal increases the TSH response (26). The enhanc-

ing effect of ClO<sub>4</sub> on endocytosis in apparently TSH refractory glands (Fig. 3 and Table II) is another strong argument in favour of this concept. Recently, partial in vivo and in vitro refractoriness of the thyroid, and particularly of hormone secretion, to repeated TSH stimuli has again been documented by Field et al. in this journal (27). The paper reviews the available literature up to 1977. A particular form of acute temporary refractoriness of endocytosis to TSH, depending on a shortage of membrane material due to a lack of exocytotic vesicles, has been described by Ekholm (28). A relationship of this phenomenon to the present observations seems rather unlikely.

Although the exact nature of the mechanisms underlying the diminished endocytotic response to TSH with increasing accumulation of iodine is not yet elucidated and the iodocompounds responsible for this interaction remain to be identified, the data reported in the present paper demonstrate that the well established chronological sequence of TSH effects on thyroglobulin neosynthesis and endocytosis in the normal gland may be changed profoundly by manipulating TSH stimulation together with iodine supply. We believe that uncoupling of the different functions of normal follicles is a key event in the pathogenesis of human colloid goiters (3, 29–31).

In order to integrate the present experiments into this concept, it should be realized that accumulation of thyroglobulin through TSH stimulation has been achieved in the present work in normal rats and mice thyroids by reversing the physiologic order of TSH response between colloid synthesis and endocytosis. The process would be greatly enhanced in thyroid glands with any kind of genuine or acquired deficiency in the endocytotic machinery. Three lines of evidence come in support of such a hypothesis. First, we have recently shown that endocytosis gradually becomes TSH refractory in most follicles of the aging mouse thyroid causing the appearance of large, distended follicles (32). Second, acquired deficiency of endocytosis as demonstrated in cold human thyroid nodules (33, 34) would seem a reasonable explanation for the involvement of large colloid filled follicles in some human goiters. Coexistence of small, hyperplastic and colloid depleted follicles with very large, colloid-filled ones within the same thyroid (3, 30) may be due, as we have recently demonstrated (31),

FIGURE 3 Thyroidal colloid content at the fourth day of TSH treatment and restoration of refractory endocytosis by a single dose of perchlorate. PAS staining.  $\times 448$ . (A) Normal rat. All follicles are rather evenly stained with PAS positive material. (B) Rat on HID and TSH for 4 d. Epithelial cells are not clearly higher than in controls. Few droplets are visible at higher magnification. (C) Rat on ClO<sub>4</sub> and TSH for 4 d. Persisting severe hyperplasia with considerably increased cell height and very little PAS positive material in the scant follicular lumina. (D) Thyroid from rat treated as in Fig. (B) except that a single dose of 50 mg NaClO<sub>4</sub> was injected i.p. together with the last TSH dose 2 h before killing. In striking contrast to the thyroids injected with TSH only, a large number of droplets are now present in most follicles.

to regionally variable metabolic disorders at the level of the follicular cells. Third, it has been observed in the hamster kept on LID (10) that ~10% of all follicles may accumulate large amounts of colloid while all other follicles become hyperplastic.

The integrity of microtubuli-microfilaments are considered essential for normal pinocytosis (35). Hence "colloid" goiters, as interpreted here, could be just another item in the fast growing list of diseases due to microtubular dysfunction (36). In the present experiments, thyroglobulin accumulated in the presence of an iodine shortage was not as poor in iodine as that extracted from some human goiters. Very low thyroglobulin iodination would presumably occur in the case of TSH-insensitive endocytosis, normal exocytosis, and simultaneous shortage of iodine.

The data presented here suggest an alternative to Marine's hypothesis that colloid goiters always result from transformation of previously hyperplastic glands (7). Although we ourselves (9), like many other authors, accepted the thesis that colloid accumulation invariably occurs as a consequence of suppression of previously high TSH secretion, it appears now that primarily colloid-rich goiters can be produced in the presence of continuously supranormal TSH stimulation. This sequence of events would require that endocytosis of colloid becomes less TSH responsive than other functions of the follicle. The present data suggest that this could occur in a TSH stimulated goiter, through intermittent availability of at least some iodine, whose organic binding sharply decreases the sensitivity of the endocytotic machinery to TSH in normal thyroid follicles. In goiter follicles, which have intrinsic disorders of metabolic pathways, the disbalanced sensitivity of single follicular functions may well be more marked (30–32).

From a clinical point of view, the familiar finding of large colloid-rich follicles as a predominant element in most simple human goiters, even in young subjects, would certainly favor the concept that excessive colloid accumulation does not require a previous stage of hyperplasia with colloid depletion, but that a slowly growing goiter in areas with moderate or intermittent iodine deficiency (37) may already be colloid rich at the time of its first appearance. Indeed, there is no clinical evidence suggesting that a hyperplastic phase would precede the evolvement of a colloid goiter.

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