Effects of Phenobarbital, Pregnenolone-16α-Carbonitrile, and Propylthiouracil on Thyroid Follicular Cell Proliferation

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Reduced thyroid hormone concentrations (T4 and/or T3) and increased thyroid-stimulating hormone (TSH) have been proposed to mediate the thyroid tumor promoting effects of hepatic microsomal enzyme inducers (MEI) and antithyroid drugs. TSH is known to stimulate thyroid gland function and growth, as well as neoplasia. Thyroid weight has been used as an indicator of thyroid gland growth in MEI studies, but little is known about the effects of these inducers on thyroid cell proliferation. Therefore, we determined the time-course of thyroid cell proliferation of rats treated with MEI, and with the antithyroid drug propylthiouracil (PTU). Male Sprague-Dawley rats were fed either a basal diet or a diet containing phenobarbital (PB) (1200 ppm), PCN (500 ppm), or PTU (30 ppm) for 3, 7, 14, 21, 30, 45, 60, or 90 days. PB and PCN treatments did not affect T3, but PTU reduced T3 60%. PB and PCN treatments reduced T4 25%, whereas PTU treatment reduced T4 90%. PB and PCN treatments increased thyroid weight 80%, and PTU increased thyroid weight 500%. TSH was not appreciably altered in PB-treated rats, but was increased 75% and 830% in PCN- and PTU-treated rats, respectively. Thyroid cell proliferation was increased 260, 330, and 850% in rats treated with PB, PCN, or PTU, respectively, for 7 days, but returned to control levels by the 45th treatment day. In conclusion, treatment with MEI that produced mild increases in TSH resulted in dramatic increases in thyroid cell proliferation, which peaked after 7 days of treatment and then returned to control values. This result is similar to that of antithyroid drugs, which produce large increases in TSH. These findings may have important implications for the role thyroid follicular cell proliferation has in mediating the thyroid tumor promoting effects of MEI.

Key words: phenobarbital, pregnenolone, propylthiouracil, thyroid hormone, cell proliferation, hepatic microsomal enzyme inducers; thyroid gland; thyroid stimulating hormone.

The hepatic microsomal enzyme inducer (phenobarbital) and several antithyroid chemicals (e.g., propylthiouracil, methylthiouracil, aminotriazole, methimazole, potassium perchlorate, and iodide) have been shown to promote thyroid tumors in rodents (Jemec, 1980; Hiasa et al., 1982a,b, 1987; Kitahori et al., 1984). The mechanism by which hepatic microsomal enzyme inducers and antithyroid drugs promote thyroid tumors has been proposed to be indirect (McClain, 1992). More specifically, it has been proposed that reduced serum thyroid hormones (T4 and/or T3) and increased serum thyroid stimulating hormone (TSH) concentrations mediate the thyroid tumor promoting effect of phenobarbital (PB) and antithyroid drugs, because TSH stimulates thyroid gland growth (Hill et al., 1989; McClain, 1989, 1992). Studies have shown that thyroid hormone replacement therapy or hypophysectomy reduces the thyroid tumor promoting effect of PB, methimazole, and methimazole, indicating the importance of TSH in the thyroid tumor promotion model (Jemec, 1980; McClain et al., 1988).

Although several antithyroid drugs promote thyroid tumors, only one hepatic microsomal enzyme inducer, PB, has been shown to increase serum TSH and promote thyroid tumors (Johnson et al., 1993; Liu et al., 1995). Pregnenolone-16α-carbonitrile (PCN) is another hepatic microsomal enzyme inducer that has been shown to increase serum TSH, however it is unknown whether PCN promotes thyroid tumors because it has not been tested in a thyroid tumor promotion model. We suspect that PCN does promote thyroid tumors because it is more effective than PB at increasing serum TSH concentration (Barter and Klaassen, 1994; Liu et al., 1995).

Central to the hypothesis of how PB, and possibly other microsomal enzyme inducers, promotes thyroid tumors is the stimulation of the thyroid gland by TSH. However, few studies have demonstrated TSH-dependent alterations in the thyroid glands of rats treated with microsomal enzyme inducers. Several studies have demonstrated that some microsomal enzyme inducers increase thyroid gland growth by measuring changes in thyroid weight (Barter and Klaassen, 1994; Liu et al., 1995; McClain et al., 1989). However, little attention has been given to thyroid follicular cell proliferation as an indication of thyroid gland growth in hepatic microsomal enzyme inducer-treated rats. To the best of our knowledge, only one study has reported the effect of PB on thyroid follicular cell proliferation, which treated rats for only 7 days (Jones and Clarke, 1993). Although an increase in thyroid follicular cell proliferation was increased in this latter study, it is unknown whether the pro-
The objectives of the present study were to determine whether hepatic microsomal enzyme inducers (PB and PCN) that have been demonstrated to increase serum TSH produce an increase in thyroid follicular cell proliferation, if so, when it occurs, and what is the duration of the proliferative response. Furthermore, we also compared the proliferative effects produced by these microsomal enzyme inducers to a chemical that produces large increases in thyroid growth, that is, the antithyroid drug propylthiouracil (PTU). Because thyroid follicular cell proliferation is known to be TSH-dependent, it was hypothesized that PB and PCN treatments increase thyroid follicular cell proliferation, which is parallel with the increase in serum TSH. Doses of PB, PCN, and PTU used in the present study were based on previous studies that showed serum TSH to be increased (Hood et al., 1999; Liu et al., 1995). Also, the dose of PB (1200 ppm) used in the present study has been shown to be large enough to promote thyroid tumors (Hiasa et al., 1985). However, it is unknown whether the dose of PCN used in the present study promotes thyroid tumors, because the thyroid tumor promoting effects of PCN have not been reported. Doses of PTU used in thyroid tumor promotion studies (1,000 ppm) are larger than in the present study (30 ppm) (Hiasa et al., 1987). Because of the extended length of the study and the strong effect PTU has on thyroid hormone homeostasis, we used the lowest dose of PTU that produces a maximal reduction in serum thyroid hormone concentrations and a maximal increase in serum TSH concentration (Hood et al., 1999).

**MATERIALS AND METHODS**

**Materials.** Propylthiouracil, 16-dehydropregnenolone, and phenobarbital, were obtained from Sigma Chemical Co. (St. Louis, MO), Pfaltz and Bauer,

### TABLE 1

<table>
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<tr>
<th>Day</th>
<th>Group</th>
<th>Body weight (g)</th>
<th>Body weight gain (g/day)</th>
<th>Feed consumption (g/kg bw/day)</th>
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<td>4.62 ± 0.44</td>
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<td>PB</td>
<td>415 ± 15</td>
<td>1.60 ± 0.173</td>
<td>61.2 ± 1.71*</td>
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*Note.* Values shown are the mean ± SE (N, 4–5 rats per group). Asterisk (*) indicates significant different from control (p < 0.05).

**FIG. 1.** Effect of PB, PCN, and PTU on liver weight. Liver weight is expressed in grams (top panel) or as grams/kg body weight (bottom panel). Symbols indicate the different feed concentrations of control (○), PB- (1200 ppm, □), PCN- (500 ppm, ▼), and PTU (30 ppm, ○)-treated rats. Each value represents the mean ± SE of 4–6 rats; *significantly different from controls (p < 0.05).
Inc. (Waterbury, CT), and Spectrum Chemical, Mfg., Corp. (Gardena, CA), respectively. 16-Dehydropregnenolone was used to synthesize pregnenolone-16α-carbonitrile as described by Sonderfan and Parkinson (1988). Bromodeoxyuridine (BrdU) and anti-BrdU antibody were obtained from Sigma Chemical Co. (St. Louis, MO). Biotinylated anti-mouse IgG (rat absorbed), and ABC Elite were obtained from Vector Labs (Burlingame, CA). Diaminobenzidine (DAB) and histomark black were obtained from Kirkgaard and Perry (Gaithersburg, MD). Lactoperoxidase (A412/A280 0.91) and glucose oxidase VII (168,200 units/gm solid) were obtained from Sigma. Radioimmunoassay (RIA) kits for total and free T4 and total and free T3 were obtained from Diagnostic Products Corp. (Los Angeles, CA). Na125I was obtained from the DuPont Company NEN Research Products (Boston, MA). Rat TSH (for radioiodinations), anti-rat-TSH serum (rabbit) and reference rat TSH were provided by the National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health (Bethesda, MD).

Animals and treatments. Male Sprague-Dawley rats (Sasco; Omaha, NE), 225–250 g, were housed in polypropylene cages containing corn-cob bedding and maintained at approximately 70°F on a 12-h light/dark cycle. Propylthiouracil (30 mg) and PB (1200 mg) were dissolved in methanol, while PCN (500 mg) was dissolved in acetone. Each dose was added to 1 kg of Purina Rodent Laboratory Chow 5001 (iodine content of 0.7 ppm), mixed thoroughly, and allowed to dry. The rats were randomly divided into 30 groups of 5 to 6 rats per group. Twenty-four groups (8 groups each) were fed PTU (30 ppm), PCN (500 ppm), or PB (1200 ppm). Six groups served as controls and received the unadulterated rodent chow. Rats were fed PTU, PCN, or PB for 3, 7, 14, 21, 30, 45, 60, or 90 days. All rats received feed and water ad libitum. Rats were monitored every 2 days by recording body weights and feed consumption. Two h prior to collecting tissues, rats were ip injected with 100 mg/kg of bromodeoxyuridine (BrdU) for quantifying thyroid follicular cell proliferation by BrdU immunocytochemistry (see procedure below).

Sampling. After the end of each treatment period, rats were lightly anesthetized with diethylether and blood was sampled from the aorta. The liver and thyroid glands were removed and weighed. Thyroid glands were fixed in 10% buffered formalin for 24 h prior to routine embedding in paraffin blocks.

Analysis of serum thyroid hormones. Approximately 1.5 ml of blood was collected in a plastic eppendorf vial. The blood was centrifuged, serum removed, and stored at –80°C. Serum total and free T4 and T3 were determined by RIA kits. The limits of detection for these kits were: total T4, 0.25 μg/dl; free T4, 0.01 ng/dl; total T3, 7 ng/dl; and free T3, 0.2 pg/ml.

Analysis of serum TSH. Rat TSH was iodinated using the glucose oxidase/lactoperoxidase method (Tower et al., 1977). Bound TSH was separated

![FIG. 2. Effect of PB, PCN, and PTU on serum total and free T3 concentrations. Serum total and free T3 over the 90-day treatment period are shown in the top and bottom panels, respectively. Symbols indicate the different feed concentrations of control (○), PB- (1200 ppm, □), PCN- (500 ppm, △), and PTU (30 ppm, ◊)-treated rats. Each value represents the mean ± SE of 4–6 rats; *significantly different from controls (p < 0.05).](image1)

![FIG. 3. Effect of PB, PCN, and PTU on serum total and free T4. Serum total and free T4 over the 90-day treatment period are shown in the top and bottom panels, respectively. Symbols indicate the different feed concentrations of control (○), PB- (1200 ppm, □), PCN- (500 ppm, △), and PTU (30 ppm, ◊)-treated rats. Each value represents the mean ± SE of 4–6 rats; *significantly different from controls (p < 0.05).](image2)
from other proliferation indices such as tritiated thymidine, PCNA, and Ki-67. Moreover, BrdU labeling-index results have been shown to be comparable to results using 10,000 cells used in mitotic index (Wynford-Thomas et al., 1982b). Further-
in-sufficient for BrdU labeling index (Jones and Clarke, 1993) as opposed to the hazards present in tritiated thymidine autoradiography and (2) a thousand cells of urinary cell proliferation, because (1) this method is absent of the radioactive chemistry was the method of choice over other methods, such as tritiated thymidine autoradiography, or mitotic index, for assessment of thyroid follicular cell proliferation (Bromley et al., 1996; Eldridge et al., 1990; Lanier et al., 1989). The labeling index (LI) was determined by counting the number of labeled nuclei, which were stained brown. One thousand nuclei (labeled plus unlabeled) of each thyroid gland were counted. To reduce bias, the following steps were performed: (1) slides were first assigned a random number, (2) each slide (containing 8 tissue sections) was viewed under a microscope and a tissue section was randomly selected, (3) a 3×3 grid (containing a total of 9 sections) within the eye piece was used to randomly select an area within a tissue section, and (4) thyroid follicular cell nuclei were then counted (labeled and unlabeled). Steps 3 and 4 were repeated until a total of 1000 nuclei were counted.

**Statistics.** Differences between control and treated animals were determined using one-way analysis of variance followed by Duncan’s multiple range post-hoc test. Significant differences between treated and control (p < 0.05) are indicated by asterisks. Statistical analyses were performed using STATISTICA 4.5, Statsoft Inc., Tulsa, Oklahoma.

## RESULTS

### Effect of PB, PCN, and PTU on Body Weight, Body Weight Gain, and Feed Consumption

PB treatment did not appreciably affect body weight or body weight gain (Table 1). Feed consumption was slightly higher in rats treated with PB for 3 (14%), 21 (41%), or 90 (19%) days as compared to controls. Pregnenolone-16α-carbonitrile (PCN) treatment did not affect body weight, weight gain, or feed consumption. Rats treated with propylthiouracil (PTU) for 14 days or longer weighed less (10 to 28%), and gained less weight (60%) than controls. Feed consumption was variable in rats treated with PTU, because it was slightly increased (13%) in rats treated for 3 or 30 days and reduced in rats treated for 21 days (16%).

### Effect of PB, PCN, and PTU on Liver Weight

Liver weight, expressed as grams (Fig. 1, top panel), was increased in rats treated with PB (30 to 70%) and PCN (30 to 60%). In contrast to PB and PCN, liver weight was reduced in rats treated with PTU (25 to 40%). When expressed as grams per kg body weight (Fig.1, bottom panel), liver weight was increased in rats treated with PB (25 to 61%) or PCN (21 to 54%), whereas it was reduced in rats treated with PTU at the 21-day (13%), 60-day (15%), and 90-day (21%) collection periods.

### Effect of PB, PCN, and PTU on Serum Total and Free T₃

Although serum total T₃ was reduced in rats treated with PB or PCN at the 3-, 7-, and 14-day collection periods (28, 34, and 16%, respectively) compared to their respective controls, serum total T₃ concentration was highly variable throughout the 90 days in the control rats (Fig. 2, top). The variation of serum total T₃ concentration among the 8 groups of control rats was greater than the reductions in serum total T₃ produced by PB or PCN treatments. In rats treated with PTU, serum total T₃ was reduced 50 to 70% at all time intervals.

Unlike serum total T₃, serum-free T₃ was increased, as compared to the respective controls, in rats treated with PCN at

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**FIG. 4.** Effect of PB, PCN, and PTU on thyroid weight. Thyroid weight is expressed as milligrams (top panel) or as mg/kg body weight (bottom panel). Symbols indicate the different feed concentrations of control (○), PB- (1200 ppm, □), PCN- (500 ppm, ▼), and PTU (30 ppm, ○)-treated rats. Each value represents the mean ± SE of 5–6 rats; *significantly different from controls (p < 0.05).
FIG. 5. Thyroid gland morphology. Representative photomicrographs of thyroid glands from control (A and E), PB (B and F), PCN (C and G), or PTU (D and H) after 7 and 90 days of treatment, respectively. Thyroid follicular cell nuclei labeled with bromodeoxyuridine are indicated with an arrow. Magnification × 140.
the 30- and 60-day collection periods. In addition, compared to their respective controls, serum-free T₃ concentration was reduced in rats treated with PB or PCN at the 14- and 21-day collection periods. Again, the variation of serum free T₃ among the control rats at the various collection periods was greater than the slight reduction in serum free T₃ produced by PB or PCN treatments (Fig. 2, bottom). PTU treatment was the most effective at reducing serum free T₃, as it was reduced at all times from 60 to 77%.

**Effect of PB, PCN, and PTU on Serum Total and Free T₄**

Serum total T₄ was reduced 19, 28, and 18% in rats treated with PB at the 3-, 7-, or 14-day collection periods, respectively (Fig. 3, top panel). Treatment of rats with PB for longer periods of time did not appreciably affect serum total T₄ concentrations. In rats treated with PCN, serum total T₄ concentrations were reduced 16 to 35%. Propylthiouracil treatment reduced serum total T₄ concentrations 54% to 90%.

In rats treated with PB or PCN, serum free T₄ concentration was maximally reduced by the 7-day collection period (40%). Serum free T₄ concentration returned to control levels in rats treated with PB at the 30-, 45-, and 60-day collection periods. Propylthiouracil treatment reduced serum free T₄ by 62% at the 7-day collection period and 96% in rats treated for 21 days or longer.

**Effect of PB, PCN, and PTU on Thyroid Weight**

Thyroid weight, when expressed as milligrams, was increased 30 to 70% and 50 to 95% in rats treated with PB or PCN, respectively (Fig. 4, top panel). Thyroid weight was increased up to 350% after 60 days of treatment, in rats treated with PTU. When expressed as milligrams per kilogram body weight (Fig. 4, bottom), thyroid weight was increased 28 to 80% in rats treated with PB or PCN. In contrast, thyroid weight was increased up to 500% after 90 days of treatment in rats treated with PTU (Fig. 4, bottom).

**Thyroid Morphology**

Figure 5 shows representative sections of thyroid tissue from control, PB-, PCN-, and PTU-treated rats after 7 and 90 days of treatment. The thyroid glands from control rats at the 7- and 90-day collection periods (A and E, respectively) contained squamous to cuboidal shaped thyroid follicular cells, medium to large colloid, and labeled nuclei. Thyroid glands from PB-treated rats at the 7- and 90-day collection periods (B and F, respectively) contained cuboidal to columnar-shaped thyroid follicular cells, small- to medium-sized colloid, and labeled nuclei. Thyroid glands from PCN-treated rats at the 7- and 90-day collection periods (C and G, respectively) contained columnar-shaped thyroid follicular cells, small colloid, and labeled nuclei. Thyroid glands from PTU-treated rats at the 7- and 90-day collection periods (D and H, respectively) contained mostly columnar-shaped thyroid follicular cells, small colloid, and labeled nuclei.

**Effect of PB, PCN, and PTU on Serum TSH Concentrations and Thyroid Follicular Cell Proliferation**

Serum TSH concentrations were not significantly increased in rats treated with PB, but were increased in rats treated with PCN (≥75% on days 7 and 14) or with PTU (up to 830% after 90 days of treatment) (Fig. 6, top panel). Thyroid follicular cell proliferation was increased in rats treated with PB, PCN, or PTU, but the increase was not sustained for the entire 90-day treatment period (Figure 6, bottom panel). For instance, in rats treated with PB, PCN, or PTU, the largest increases in thyroid follicular cell proliferation occurred in rats treated for 7 days (260, 330, and 850%, respectively). Thyroid follicular cell proliferation was increased less or not at all in rats treated with PB, PCN, or PTU for longer periods of time (Figure 6, bottom panel).
DISCUSSION

In the present study, serum total and free T₄ concentrations were reduced in rats treated with PB or PCN, whereas serum total and free T₃ concentrations were not reduced significantly below the normal variation in control rats (Figs. 2, 3). These results are consistent with previous studies (Barter and Klaassen, 1994; Liu et al., 1995). In the present study, PTU was much more effective at reducing serum T₄ and T₃ concentrations than PB and PCN (Figs. 2, 3), and is probably due to inhibition of thyroid gland synthesis of T₄ and T₃ by PTU (Engler et al., 1982; Kieffer and Larsen, 1991; Shiroozu et al., 1983). In contrast to PTU, PB and PCN act by an extrathyroidal mechanism to reduce serum T₄ concentration (Barter and Klaassen, 1992b). The extrathyroidal mechanism by which PB and PCN reduce serum T₄ is by increasing hepatic T₄ glucuronidation and elimination (Barter and Klaassen, 1992a; De Sandro et al., 1991; Japundzic et al., 1976; Liu et al., 1995; McClain et al., 1988). Consistent with this extrathyroidal mechanism, liver weight was increased in PB- and PCN-treated rats in the present study (Fig. 1).

It is unclear why PB and PCN do not reduce serum T₃ concentration. One possibility is that compensatory mechanisms that maintain serum T₄ and/or T₃ may have attenuated the effects of PB and PCN on serum thyroid hormone concentrations. For instance, PB treatment for 3, 7, or 14 days reduced serum total and free T₃ concentrations, but not in rats treated with PB for longer periods of time (Fig. 3). This result may suggest that a compensatory mechanism was activated in PB-treated rats that reversed the imbalance in T₃ homeostasis. A compensatory mechanism could also have been activated in PCN- or PTU-treated rats, but was less effective at reversing the imbalance in T₃ homeostasis compared to rats treated with PB. In addition, a compensatory mechanism may also explain why serum T₃ concentrations were not reduced as much as serum T₄ concentrations in rats treated with PB, PCN, or PTU. Compensatory mechanisms that may maintain serum concentrations of T₄ and/or T₃ include: increased synthesis of T₄ and T₃ by the thyroid gland, due to TSH stimulation; increased T₃ synthesis in extrathyroidal tissues by outer-ring deiodination; recovery of T₄/T₃ from T₄-SO₄/T₃-SO₄ by sulfatases; and/or increased enterohepatic circulation (de Herder et al., 1989; Kohrle, 1990, 1991; Rutgers et al., 1989; Visser et al., 1988).

PCN and PTU treatments, but not PB, produced statistically significant increases in serum TSH in the present study (Fig. 6, top panel). Although it is difficult to conclude whether TSH mediated the increase in thyroid gland observed in the present study, it is well accepted that TSH stimulation results in increases in thyroid gland weight and thyroid follicular cell proliferation. For instance, in vitro studies have demonstrated that TSH stimulates thyroid follicular cell proliferation (Smith et al., 1986). Also, increases in thyroid follicular cell proliferation produced by aminotriazole treatment has been shown to be specific to TSH stimulation (Smith et al., 1987). Previous studies have shown TSH to be mildly increased in rats treated with PB (Barter and Klaassen, 1994; Liu et al., 1995). Recently, we have shown that thyroid follicular cell proliferation is very sensitive to serum TSH stimulation, where small increases in serum TSH results in large increases in thyroid follicular cell proliferation (Hood et al., 1999). Therefore, it is possible that PB produces a small increase in serum TSH, albeit statistically insignificant, that stimulates thyroid follicular cell proliferation. Alternatively, it is also possible that PB may act as a direct mitogen to thyroid follicular cells. These possible explanations require further study.

In the present study, thyroid gland weight (Fig. 4) and thyroid follicular cell proliferation (Fig. 6, bottom panel) were increased in rats treated with PB, PCN, or PTU, suggesting that thyroid gland growth was stimulated. Although a previous study has shown thyroid follicular cell proliferation to be increased in rats treated with PB for 7 days, (Jones and Clarke, 1993), this is the first study to show that thyroid follicular cell proliferation peaks after 7 days of treatment with PB, PCN, or PTU, and then returns to control levels. This result is strikingly similar to those obtained by Wynford-Thomas et al. (1982b).

These investigators have shown that treatment of rats with the antithyroid drug aminotriazole (ATA) results in maximal levels of thyroid follicular cell proliferation after 7 days of treatment, which then returns to control levels. The similar proliferative response between the present study and the latter cannot be entirely attributed to similarities in experimental design. Not only did we include a different class of drug (two hepatic microsomal enzyme inducers), but we also assessed thyroid follicular cell proliferation using BrdU labeling indices, whereas the previous study by Wynford-Thomas et al. (1982b) assessed thyroid follicular cell proliferation by mitotic indices. Although we used different methods, we obtained similar results, not only demonstrating the reproducibility of the proliferative response but also that BrdU labeling indices is comparable to traditional proliferation assessment methods.

Thyroid follicular cell proliferation did not correlate with thyroid gland growth (i.e., thyroid gland weight) in the present study. However, morphological changes of thyroid tissue from rats treated with PTU did appear to correlate with increases in thyroid growth, as these rats had the largest increases in thyroid weight (Fig. 4), the largest increases in cellular hypertrophy, and the largest reductions in colloid space (Fig. 5). These results support the finding of Wynford-Thomas et al. (1982b) that thyroid follicular cell proliferation dissociates from thyroid gland growth in rats constantly stimulated with TSH. It has been hypothesized that the decline in thyroid follicular cell proliferation is due to desensitization of thyroid follicular cells to the mitogenic actions of TSH (Smith et al., 1987; Stringer et al., 1985; Wynford-Thomas et al., 1982a, 1983). Details of the growth-desensitization mechanism have not been fully elucidated.

In conclusion, thyroid follicular cell proliferation is increased in rats treated with the hepatic microsomal enzyme
inducers PB and PCN, in addition to antithyroid drugs such as PTU. Furthermore, the increases in thyroid follicular cell proliferation of PB-, PCN-, and PTU-treated rats occur within the first week of treatment and then return to control levels. Further studies investigating the dose-dependent effects of microsomal enzyme inducers on thyroid follicular cell proliferation is essential.

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REFERENCES


