

Adrenal Atrophy and Thyroid Inhibition Following PABA.* (26161)

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Atrophy of the adrenal gland following goitrogen administration has been suggested to occur either as a result of a mechanism related to thyroid inhibition or a direct action on the adrenal gland(1). Results of testing various goitrogens showed that all treatments which produced adrenal involution, did so with concomitant thyroid hypertrophy except for administration of p-aminobenzoic acid (PABA)(2). Since the possibility was suggested that PABA treatment influenced the adrenal gland by an extra-thyroidal action (2), this study was undertaken to evaluate thyroid and adrenal gland activities in PABA-fed rats. Since studies(3,4,5) have shown that uptake and incorporation of radioactive phosphorus into the inorganic phosphorus of the adrenal gland vary with conditions affecting the activity of the gland, an attempt was made to assess adrenal activity based on P³² uptake. The accumulation of I¹³¹ in the thyroid gland was taken as an index of glandular activity(6).

Methods. Groups of Sprague-Dawley female rats, average body weights 190 g, were housed 5 to a cage and given ground Purina laboratory chow and tap water *ad libitum*. The experimental groups were fed 2% PABA[‡] in the diet for periods of 2, 4 and 12 weeks, and one group was fed 4% PABA in the diet for 12 weeks. Control animals were placed on the ground chow diet alone. Twenty-four hours before termination of an

experiment, each rat was given a single intraperitoneal injection of 5 μ c of I¹³¹. On the next day, 2 hours before killing, each animal was given a single intraperitoneal injection of 100 μ c of P³². The animals were killed by decapitation so as to leave the thyroid gland intact. The thyroid and adrenal glands were removed and weighed to the nearest 0.1 mg and the values expressed as milligrams of tissue per 100 g body weight. The thyroid glands were placed in 3 ml of an alkaline KI digestion solution(6) and heated in a boiling water bath until digestion was complete. The adrenal glands were placed in a grinding tube and ground with 5 ml trichloroacetic acid. One aliquot of the adrenal preparation was used for determination of P³² and the other was used to determine inorganic phosphorus content(7).

Aliquots from the thyroid and adrenal gland preparations and samples of I¹³¹ and P³² and mixtures of the two, in known concentrations, were counted using a well-type scintillation counter and an end window Geiger-Müller tube. From the data obtained for the standard preparations of I¹³¹ and P³², simultaneous equations were derived to express the counting ratios of both isotopes in both types of detectors. Using these equations and the counting rates of the tissue samples, radioactivity of I¹³¹ in the thyroid gland and of P³² in the adrenal gland was determined. Level of activity was recorded as counts per minute per gland (c/m/gland) and as counts per minute per 100 mg gland (c/m/100 mg) to indicate total and relative isotope uptake of the tissues.

Results. Feeding rats 2% PABA for 2 weeks failed to produce a significant change in weights of thyroid or adrenal glands (Table I). The thyroid glands from animals given PABA for 4 weeks showed an apparent enlargement when compared to the comparable control group. However, the thyroid gland weights of the 4 week control group

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[‡] Samples of PABA supplied through the courtesy of S. B. Penick Co., Jersey City, N. J.

TABLE I. Effect of PABA Administration on Adrenal and Thyroid Weight of Female Rats.

	No. animals	Body wt, g	Adrenal wt		Thyroid wt	
			mg	mg/100 g body wt	mg	mg/100 g body wt
2 wk treatment						
Control	5	204 ± 7.8	64.5 ± 1.1	31.7 ± 4.9	13.8 ± 3.8	6.8 ± 1.8
PABA 2%	5	213 ± 8.0	58.5 ± 5.7	27.6 ± 3.4	15.8 ± 2.5	7.5 ± 1.4
4 wk treatment						
Control	5	211 ± 7.3	59.0 ± 5.5	27.0 ± 2.3	9.4 ± 1.9	4.5 ± .9
PABA 2%	5	231 ± 19.2	57.0 ± 2.5	24.7 ± 1.8	16.0 ± 3.5*†	6.9 ± 1.2*†
12 wk treatment						
Control	7	246 ± 31.4	55.9 ± 5.3	23.0 ± 3.4	12.1 ± 1.8	5.1 ± 1.1
PABA 2%	7	245 ± 13.2	48.2 ± 5.3*	19.7 ± 2.4	11.8 ± 2.6	4.9 ± 1.2
PABA 4%	8	220 ± 8.1	43.6 ± 6.6*	18.7 ± 3.5*	19.6 ± 7.7*	8.8 ± 3.5*

* P < 0.05.

† Significant only in relation to comparable control. Not significant in comparison to other control values.

were somewhat low and the comparison of thyroid glands of 4 weeks PABA treated rats to the 2 week controls revealed no significant difference ($P > 0.05$). The only definite thyroid hypertrophy was seen in the rats administered 4% PABA for 12 weeks where adrenal involution also was evident. In the group given 2% PABA for 12 weeks, absolute adrenal weight was significantly reduced from control weight though relative size of the gland was not different from that of the controls ($P > 0.05 < 0.10$). Thyroid glands of this latter group were of a size comparable to control glands. In no case was the body weight of a treated group changed from control weights.

The I^{131} level in thyroid glands of PABA-treated rats gave an indication of reduced glandular activity (Table II). While total I^{131} content in thyroid glands from rats given 2% PABA for 2 or 4 weeks was unchanged from the normal, a significant reduction was seen in the isotope uptake per unit gland weight. After the 12 week treatment interval, both experimental groups showed a marked decrease in total and relative thyroidal accumulation of I^{131} . Though adrenal glands from both treated groups showed a reduction in total inorganic phosphorus level, concentration per 100 mg tissue was normal. Only rats fed 4% PABA for 12 weeks were found to have a decrease in P^{32} uptake per

TABLE II. Effect of PABA on Thyroidal I^{131} and Adrenal Inorganic Phosphorus and P^{32} in Female Rats.

	Thyroidal I^{131}		Adrenal inorganic phosphorus		Adrenal P^{32}	
	c/m/gland	c/m/100 mg	μg/gland	μg/100 mg	c/m/gland	c/m/100 mg
	$\times 10^3$					
2 wk treatment						
Control	128 ± 34.9	1006 ± 422.0	23.9 ± 3.9	37.4 ± 4.3	8645 ± 1724	13371 ± 932
PABA 2%	91 ± 18.4	587 ± 159.6*	21.6 ± 3.0	37.0 ± 5.5	8072 ± 1183	13662 ± 2277
4 wk treatment						
Control	123 ± 35.9	1296 ± 252.7	21.4 ± 3.1	35.7 ± 1.9	7907 ± 907	13288 ± 1396
PABA 2%	87 ± 25.2	544 ± 166 *	19.9 ± 2.6	35.0 ± 5.0	11910 ± 5599	21043 ± 10464
12 wk treatment						
Control	226 ± 25.2	1997 ± 40.9	22.2 ± 3.2	40.6 ± 5.3	1150 ± 439†	2094 ± 781
PABA 2%	119 ± 18.2*	1142 ± 42.8*	16.9 ± 3.2*	36.8 ± 2.4	756 ± 403	1670 ± 628
" 4%	113 ± 58.0*	474 ± 81.2*	14.9 ± 1.2*	32.6 ± 2.6	595 ± 106*	1315 ± 334

* P < 0.05.

† Conditions required that these samples be counted using a different G-M Counter resulting in a lower counting rate. All samples from this treatment period were counted under the same conditions.

adrenal gland, and in these rats also the level of P^{32} per unit gland weight was unchanged from control values.

Discussion. The possibility of an extra-thyroidal action of PABA has previously been suggested since the adrenal atrophy in rats after treatment with 2% PABA was not accompanied by thyroid hypertrophy but was associated with an unusual response of the plasma adrenocorticoid concentrations(2). In comparison, the results reported here indicate that rats fed 2% PABA for 12 weeks show at most only a suggestion of adrenal involution. In these animals, the absolute adrenal weight was reduced but decrease in relative weight was not significant ($P > .05 < .10$). In any event, definite thyroid inhibition was evident in groups treated with the goitrogen for 12 weeks as indicated by the decreased I^{131} uptake in both groups and the thyroid hypertrophy in the animals given 4% PABA. Additional indications of thyroid inhibition were seen in the decrease in I^{131} level in thyroid glands of rats treated for 2 and 4 weeks with PABA. Thus the results indicate that inhibition of the thyroid gland accompanies and precedes adrenal atrophy induced by PABA administration.

Other data fail to indicate a direct action of this goitrogen on the adrenal gland since administration of a single dose of PABA to rats failed to bring about depletion of adrenal ascorbic acid or cholesterol(8). Conditions which affect adrenal gland activity have been associated with changes in incorporation of P^{32} into the inorganic phosphorus of the gland(3,4,5). In the present study, the decrease in inorganic phosphorus and P^{32} in the adrenal glands of PABA-treated rats appears to be related to reduced gland size rather than to changes in activity.

Though the possibility of a direct action of

PABA on the adrenal gland cannot be excluded, these data do establish that thyroid inhibition occurs under conditions which produce adrenal involution associated with PABA treatment. Therefore PABA administration could induce adrenal involution by a mechanism associated with thyroid inhibition alone or coupled with a direct action of the adrenal gland(1,2).

Summary. The results clarify a previous report(2) in which the possibility of a direct action of PABA on the adrenal gland was suggested as a more likely mechanism for induction of adrenal atrophy by this goitrogen. These data indicate that under conditions where administration of PABA produced adrenal atrophy, inhibition of the thyroid gland occurred as indicated by reduced thyroidal I^{131} uptake. Thus further evidence is presented to associate goitrogenic activity with ability to induce adrenal atrophy, though the possibility of a direct action of PABA on the adrenal gland coupled with thyroid inhibition can not be excluded.

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