A Mode of Action for Thyroid Inhibition by Reserpine.* (25168)

RICHARD C. MOON† AND CHARLES W. TURNER
Dept. of Dairy Husbandry, University of Missouri, Columbia

Reserpine administration has been reported to depress O_2 consumption in rats(1) and guinea pigs(2). Other studies have shown reserpine to decrease thyroidal-I^{131} uptake(3, 4), release rate(5) and thyroxine secretion rate(5). Little is known, however, of the mechanism by which reserpine exerts its antithyroid activity. It has been suggested the drug inhibits thyroid function by 1) inactivation of thyroxine(1,2) or thyrotropin (TSH), 2) inhibition of organic-binding I_2(3,4) or 3) suppression of TSH secretion through inhibition of hypothalamic centers(5,6). In the present study, we attempted to determine which of the suggested mechanisms is responsible for the antithyroid activity of reserpine in the rat.

Materials and methods. Young mature female rats of Sprague-Dawley-Rolfsmeyer strain weighing 190-230 g were housed in a room artificially illuminated during daylight hours and at uniform temperature of 79 ± 1°F. They were allowed free access to Purina Lab Chow and water. *Exp. 1.* Fifteen rats were injected i.p. with 2 μc carrier-free I^{131}. Forty-eight hours were allowed for I^{131} fixation by thyroid gland. External neck counts were made at this time and every 24 hours thereafter by first anesthetizing each animal with ether, then placing it on a lead plate with neck resting on a scintillation probe containing a 2″ NaI crystal. Care was taken in placement of the animal to insure the same geometrical relationship at each successive counting period. Thyroidal radioactivity was measured with scintillation counter, Nuclear-Chicago (N.C.) Model DS5, connected to rate meter, N.C. Model 1620A. Conventional corrections were made for background and radioactive decay. Neck counts were made on days 2-4 after initial count to establish that thyroidal-I^{131} output was proceeding normally. Each rat received daily subc. injections of 2.5 μg/100 g l-thyroxine, to block endogenous TSH secretion, .2 unit TSH‡ and 10 μg/100 g reserpine beginning on days 4, 7 and 10, respectively, after initial count and continuing throughout experimental period. Thyroidal-I^{131} release curve was constructed by plotting average percent dose against time in days. Average hourly release thyroidal-I^{131} (k′4) was calculated by the method of Brownell(7). *Exp. 2.* Fifty-nine additional female rats were injected daily with 10 μg/100 g reserpine and/or 4 mg/100 g 1-methyl-2-mercaptoimidazole (tapazole) for 10 days. Sixteen served as controls of which half were injected with .1 ml/100 g distilled water/day. Body weight of each rat was recorded daily. Twenty-four hours after last injection, each animal was weighed and killed with ether. Pituitary, thyroid and adrenal glands were removed rapidly, blotted to remove surface moisture and weighed to nearest .1 mg on a Roller-Smith Torsion balance.

Results. Administration of reserpine did not significantly affect release of thyroidal-I^{131} resulting from daily injections of TSH (Fig. 1). Average hourly release thyroidal-I^{131} (k′4) during period of TSH injections was .00489 ± .00059 as compared with .00529 ± .00040 during period in which both TSH and reserpine were administered. Injections of tapazole and/or reserpine for 10 days did not significantly affect pituitary or adrenal weight (Table I). Thyroid weight, however, was significantly increased over controls in animals treated with tapazole but concomitant administration of reserpine prevented the increase in thyroid weight which resulted from injections of tapazole. Thyroid weight of reserpine-treated rats did not differ significantly from that of controls.

* Contribution from Dept. of Dairy Husbandry, Mo. Agr. Exp. Sta. Journal Series No. 2093. Approved by the Director.

† Postdoctoral Fellow of NIH. Investigation supported in part by research grants from P.H.S.

‡ We are indebted to Jensen-Salsbery Labs., Kansas City, Mo., for TSH and to Eli Lilly and Co., Indianapolis, Ind. for reserpine and tapazole.

TABLE I. Effect of Reserpine and/or Tapazole upon Gland Weight of Female Rats.

Treatment for 10 days	No. of rats	Avg Body wt (g)	Avg Thyroid (mg/100 g)	Avg Pituitary (mg/100 g)	Avg Paired adrenals (mg/100 g)
Control	16	208.3	3.86 ± .18*	4.56 ± .13	25.2 ± .53
Reserpine (10 μg/100 g/day)	15	218.2	4.22 ± .77	4.46 ± .19	23.5 ± 1.34
Tapazole (4 mg/100 g/day)	14	205.0	7.86 ± .34†	4.81 ± .21	25.6 ± 1.75
Reserpine + tapazole	14	219.9	4.42 ± .21	4.74 ± .19	24.0 ± .76

* Stand. error of mean.

† Significant at .1% level from control.

Discussion. It is generally accepted that goitrogenic compounds depress thyroid function by inhibiting organic-binding of I_2 which, in turn, decreases the circulating thyroxine level with a subsequent increase in TSH secretion and enlargement of the thyroid. It is also well known that goitrogen administration results in an apparent increase in thyroidal-I^{131} release rate since the thyroid is unable to hold recycled I^{131} which is released upon degradation of radioactive hormone secreted by the thyroid gland. Reserpine, on the other hand, has been shown to depress thyroidal-I^{131} release rate(5). This suggests that some factor, other than inhibition of organic-binding, is responsible for the antithyroid activity of the drug. Results of the present experiment are consistent with this interpretation. Tapazole injections resulted in a significant increase in thyroid weight over that of controls, however, concomitant administration of reserpine prevented thyroid weight increase. If the mode of action of reserpine was similar to that of goitrogens, it would be expected that thyroid weight would increase rather than decrease when tapazole and reserpine were injected together or, at least, thyroid enlargement should have been evident in animals treated with reserpine alone. These data indicate a suppression of TSH secretion or inactivation of the hormone so that it is ineffective at the thyroid level. The former suggestion appears the more plausible since thyroidal-I^{131} output resulting from exogenous TSH was not affected by reserpine injections (Fig. 1). If inactivation of TSH had occurred, thyroidal-I^{131} release rate would have been depressed since endogenous TSH secretion was effectively blocked by injections of thyroxine. Recent studies, employing enzyme activity of the thyroid as an index of thyroid function, also indicate a reduced TSH secretion upon administration of reserpine(6).

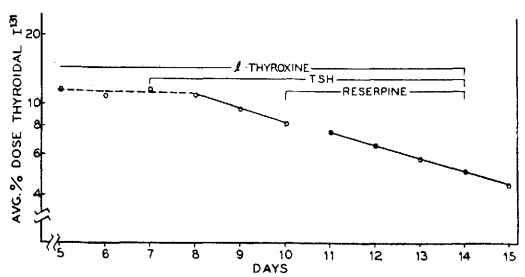

FIG. 1. Effect of exogenous thyroxine, TSH and reserpine upon thyroidal-I^{131} release.

It has also been suggested that reserpine antagonizes thyroxine since the drug prevents increased O_2 consumption which normally results from administration of thyroxine(1,2). Data of the present study are not in accord with this concept since reserpine administration did not increase thyroidal-I^{131} release rate. If the drug antagonizes or inactivates thyroxine, an increase would be expected since the thyroxine block of endogenous TSH secretion would be, at least partially, removed thus resulting in an elevated TSH level and subsequent increased output of thyroidal-I^{131}. It appears, therefore, that the antithyroid activity of reserpine may be attributed to a reduction in TSH secretion *via* the hypothalamo-hypophyseal system since it has been shown reserpine exerts a depressant effect upon neural centers of this area(8,9).

Summary. Reserpine had no significant effect upon thyroidal-I^{131} release rate resulting from injections of thyrotropin (TSH) into young mature female rats whose endogenous secretion of TSH was effectively blocked with thyroxine. In another experiment, reserpine prevented thyroid enlargement which normally results from administration of the goitrogen tapazole. Injections of tapazole and/or reserpine did not significantly affect pituitary or adrenal weight. It appears reserpine

inhibits thyroid function in the rat through suppression of TSH secretion.

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Received July 10, 1959. P.S.E.B.M., 1959, v102.

Effect of Azo-Dye Carcinogenesis on Hexosamine Synthesis in Rat Liver.* (25169)

DONALD E. KIZER AND THOMAS A. MCCOY
Biomedical Division, Samuel Roberts Noble Fn., Ardmore, Okla.

The enzyme which catalyzes formation of glucosamine from hexose-6-phosphate and glutamine was first observed in extracts of *Neurospora crassa*(1) and was subsequently demontrated in pig kidney extracts(2), rat liver extracts(3,4) and several tissues of the growing rabbit(5). Recently, hexosamine synthesis was demonstrated in a spectrum of transplantable tumors and it was observed that hexosamine synthesis by transplanted azo dye-induced hepatomas significantly exceeded the synthesis observed with normal liver(6). It was concluded from these observations that either carcinogenesis, subsequent transplantation or both, resulted in increased production of amino sugars by hepatomas when compared to the tissue of origin(6). The purpose of this investigation was to measure the extent of amino sugar synthesis in liver during azo-dye carcinogenesis to ascertain whether the increased amino sugar synthesis was associated with changes occurring during carcinogenesis or transplantation.

Materials and methods. Throughout an experimental period of ca. 150 days, female Holtzman rats were maintained on an *ad lib.* semi-synthetic, riboflavin-deficient diet described by Medes, *et al.*(7). The diet for half the animals contained 0.06% 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) during the first 90 days of the experiment.† Five animals from each group were sacrificed at 30-day intervals during the first 90 days. Then the dye was withdrawn from the diet and dye-fed animals were sacrificed individually when hepatomas were detected by palpation. Five control animals were sacrificed following last determination on dye-fed animals. Livers from both groups were perfused with cold isotonic saline, pooled, homogenized in 0.1 M phosphate buffer(9) pH 6.6, and diluted with buffer to give a tissue nitrogen content of 4 to 5 mg/ml in the reaction mixture. The reaction mixture contained 0.03 M glutamine, 0.02 M glucose-6-phosphate, phosphate buffer (pH 6.6) and homogenate. The conditions just described were optimum for hexosamine synthesis by liver homogenates(6). Aliquots were removed at 0 and 180 minutes and analyzed for total hexosamine content by the Blix(10) modification of the Elson-Morgan method(11). Tissue nitrogen content was determined by Kjeldahl procedure(12).

Results. Glucosamine synthesis appeared the same in livers of both groups throughout the 90-day azo-dye regimen, Table I. Pathological examinations‡ of the precancerous liv-

* The authors express their appreciation to Julia Wheeler and Boyd Howell for technical assistance.

† 3'-Me-DAB was synthesized by the method of Giese, *et al.*(8). The authors are grateful to Merle Maxwell for preparing 3'-Me-DAB.

‡ The authors are indebted to Dr. E. S. Irvine for pathological examinations.