Summary. A study of the effect of pepsin, carboxypeptidase, trypsin and chymotrypsin on the oxytocic activity of extracts of the rat hypothalamus and posterior lobe of the hypophysis. Both extracts show similar behavior. Chymotrypsin is the only enzyme that produces complete or almost complete loss of the oxytocic activity. No decrease in oxytocic activity is produced by the other enzymes. Incubation of the extracts at pH 7.3 without enzymes results in an appreciable loss of oxytocic activity by both extracts.

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Antithyroid Properties of 2-Amino, 5-Nitrothiazole in Rats.* (22069)

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The report that 2-amino, 5-nitrothiazole caused the complete suppression of sexual manifestations and functions in fowl(1-3) has been explained as being the result of inhibition of secretion of pituitary gonadotropin(3). Although the effect of the compound appeared to be restricted to fowl(3), interest in a possible antigonadotropic substance that is not related to the sex-hormones has prompted the present investigation of 2-amino, 5-nitrothiazole utilizing the rat as the experimental animal.

Materials and methods. Eighteen litters of Long-Evans female rats from 45 to 49 days of age were assigned to 4 groups so that each group contained 18 rats from different litters and so that each group had the same average body weight. At this time 2 groups were ovariectomized and 2 groups were sham-operated. One operated group and one sham-operated group was started the next day on commercial 2-amino, 5-nitrothiazole† added to the diet at a concentration of 0.3%. All other rats received the same diet (commercial laboratory chow‡ that is adequate in iodine) with no drug added. All rats were maintained on their respective diets and tap water *ad libitum* until sacrifice 30 days later. The intact animals were given a 2 μc tracer dose of carrier free I^{131}§ by the intraperitoneal route and sacrificed 24 hours later. The operated animals were handled exactly as above except that they were sacrificed 48 hours post I^{131} administration. At sacrifice the body, pituitary, thyroid, adrenal and uterus weights were recorded and the thyroid glands were placed in 2 cc of Bouin's fluid and assayed for radioactivity in a well-type scintillation counter against 3 aliquots of the original I^{131} as prepared for injection. The radioactivity measurements were expressed as the percentage of injected dose found in the sample and the counting statistics were such that the resulting probable error was less than 1%. The thyroid glands were then sectioned and stained for histological study. In another experiment 30 day-old male Sprague-Dawley rats were assigned to 3 groups of 15 animals so that the average body weight of all groups was the same. These rats were maintained

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† "Enheptin", a commercial product containing aminonitrothiazole not less than 20%, soybean oil meal, not more than 80%, crude protein not less than 33%, crude fat not less than 0.3% and crude fiber not more than 7%, sold by American Cyanamid Co. (Lederle Laboratories Division), New York City.

‡ Purina Laboratory Chow. Ralston Purina Co., St. Louis, Mo.

§ Obtained from Oak Ridge National Laboratory.

TABLE I. Effect of Feeding 2-Amino, 5-Nitrothiazole (0.3% of Diet Adequate in Iodine for 30 Days) to Intact and Ovariectomized Female Long-Evans Rats on the Body Weight, Adrenal Weight, Thyroid Weight, Pituitary Weight, Uterus Weight and Percent of Administered I^{131} Accumulated by the Thyroid Gland (24 Hr between Isotope Administration for Intact Animals and 48 Hr for Ovariectomized Animals). Mean ± stand. error reported.

Treatment	No. of animals	Body wt (g)	Adrenal (mg)	Thyroid (mg)	Pituitary (mg)	Uterus (mg)	I^{131} (%)
Intact	18	154 ± 4	40.3 ± 2.2	9.2 ± .5	6.20 ± .24	181 ±23	6.35 ± .48
Intact + 2-amino, 5-nitrothiazole	18	142*± 4	31.8†± 1.7	11.1†± .4	5.96 ± .24	141 ±17	3.12†± .27
Ovariectomized	17	161 ± 4	38.6 ± 2.0	9.6 ± .5	7.35 ± .20	40 ± 3	4.39 ± .30
Ovariectomized + 2-amino, 5-nitrothiazole	16	144*± 4	31.3†± 1.3	11.4†± .5	6.84 ± .26	35 ± 2	.60†± .11

* P_{05} when tested by t-test against mean immediately above.
† P_{01} " " " " " " " " .

TABLE II. Effect of Feeding 2-Amino, 5-Nitrothiazole (0.30%) or Purified 2-Amino, 5-Nitrothiazole (0.06%) in a Diet Low in Iodine to Male Sprague-Dawley Rats for 8 Days on Body Weight, Testis Weight, Thyroid Weight, Pituitary Weight, and Percent of Administered I^{131} Accumulated by the Thyroid Gland (18 Hr between Isotope Administration and Sacrifice). 15 animals in each series.

Treatment	Body wt (g)	Testis (g)	Thyroid (mg)	Pituitary (mg)	I^{131} (%)
.06% 2-amino, 5-nitrothiazole	91 ± 1	1.21 ± .04	7.5 ± .3	3.86 ± .12	17.1 ± 1.6
Low iodine	92 ± 2	1.22 ± .32	6.6*± .2	3.84 ± .22	25.4†± 1.6
.30% 2-amino, 5-nitrothiazole	92 ± 2	1.16 ± .18	8.1†± .4	3.82 ± .10	16.7†± .9

* † Same as in Table I.

on a diet low in iodine‖ for 8 days(4). One group received commercial 2-amino, 5-nitrothiazole added to the diet at a concentration of 0.30%, another group received the purified compound¶ at a concentration of 0.06%, while one group received no drug treatment. All animals were maintained on these diets and tap water *ad libitum* until sacrifice. Eighteen hours before sacrifice each rat received a tracer of I^{131} and radioactivity of the thyroid gland determined as before.

Results. The feeding of a commercial preparation of 2-amino, 5-nitrothiazole (0.30% of a diet adequate in iodine) for 30 days to intact or ovariectomized Long-Evans rats had no effect on pituitary, or uterus weights. The 2-amino, 5-nitrothiazole treated rats had smaller body and adrenal weights, larger thyroid weights, and lower 24 hour and 48 hour thyroidal accumulation I^{131} values than non-treated rats (Table I).

Male Sprague-Dawley rats fed either a commercial preparation of 2-amino, 5-nitrothiazole or a purified preparation of the compound (0.30% or 0.06% respectively of a diet poor in iodine) for 8 days had no effect on body, testis or pituitary weights. However, the 2-amino, 5-nitrothiazole treated rats had larger thyroid glands and a lower 18-hour thyroidal accumulation of I^{131} than the non-treated rats (Table II).

In all groups treated with 2-amino, 5-nitrothiazole the thyroid glands gave a histological picture of columnar follicular cells and less densely staining colloid that is consistent with effects given by treatment with antithyroid compounds of the thiouracil type (Fig. 1, 2).

Discussion. 2-amino, 5-nitrothiazole had no effect on gonad function, as judged by testis weights or uterus weight, when administered to male or female rats. This is in agreement with previous reports(3) since the effectiveness of 2-amino, 5-nitrothiazole in suppressing gonad function was limited to fowl and could not be extended to mammals.

‖ Remington Diet No. 348. Each 100 g contains: wheat gluten, 16 g; dried yeast, 4 g; corn meal 78 g; $CaCO_3$, 1 g; and NaCl, 1 g.

¶ 2-amino, 5-nitrothiazole, Lot No. 251T2F, assay 97.2% pure, contributed by Lederle Laboratories.

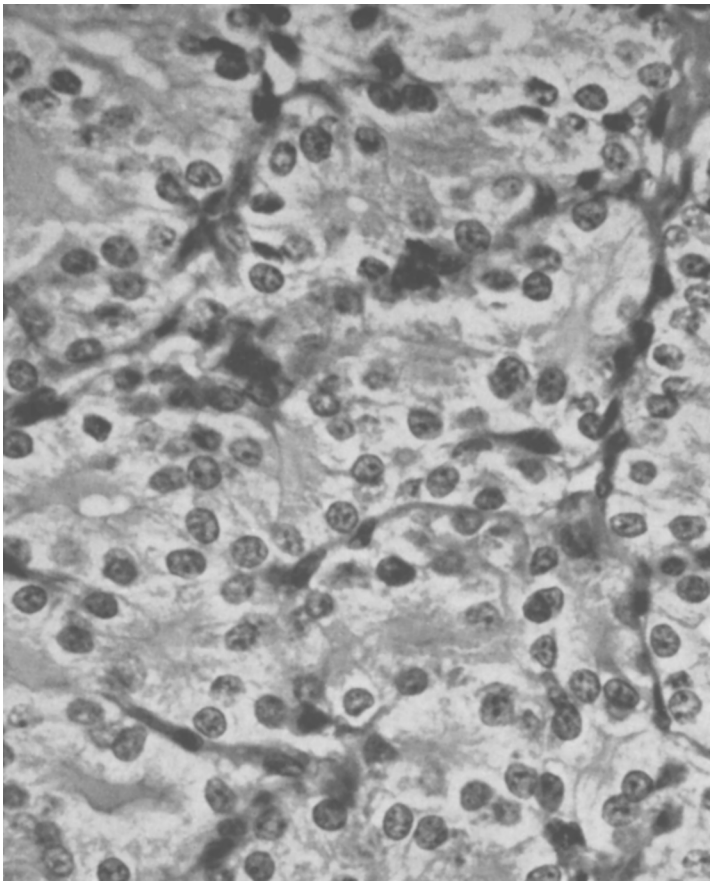

FIG. 1. Thyroid gland from female Long-Evans rat that had been fed 0.30% 2-amino, 5-nitrothiazole for 30 days. Bouin fixation, hematoxylin and eosin. (×600.)

However, there seems to be little doubt that 2-amino, 5-nitrothiazole had a definite antithyroid action in these rats since the thyroid weight and follicular cell height was increased and the thyroidal accumulation of I^{131} was lowered in rats treated with the compound. It is not surprising that 2-amino, 5-nitrothiazole possesses antithyroid properties since it is well known, in man at least, that aminothiazoles possess definite antithyroid properties that produce an increase in thyroid weight and a lowering of I^{131} uptake. In fact, the aminothiazoles have been used for the control of hyperthyroidism(5). It seems likely that the other changes noted in the aminonitrothiazole-treated rats, *i.e.*, a depressed body and adrenal weight, might also be ascribed to an induced hypothyroidism since these changes are not inconsistent with changes produced by hypothyroidism(6). Indeed, it is possible that the suppression of sexual function in fowl noted following treatment with the compound might also, at least in part, be ascribed to a relative lack of thyroid hormone since various degrees of hypothyroidism may contribute to a lack or regression of gonad function and the anterior pituitary responses to changes in gonad function(7). The fact that the gonadal suppression noted in 2-amino, 5-nitrothiazole treated fowl has not been attributed to a decreased thyroid function does not detract from the foregoing suggestion because the only tests reported on thyroid function in fowl treated with 2-amino, 5-nitrothiazole were tests designed to show if the compound suppresses the pituitary production of thyrotropin and were not designed to test the possibility of a direct action of 2-

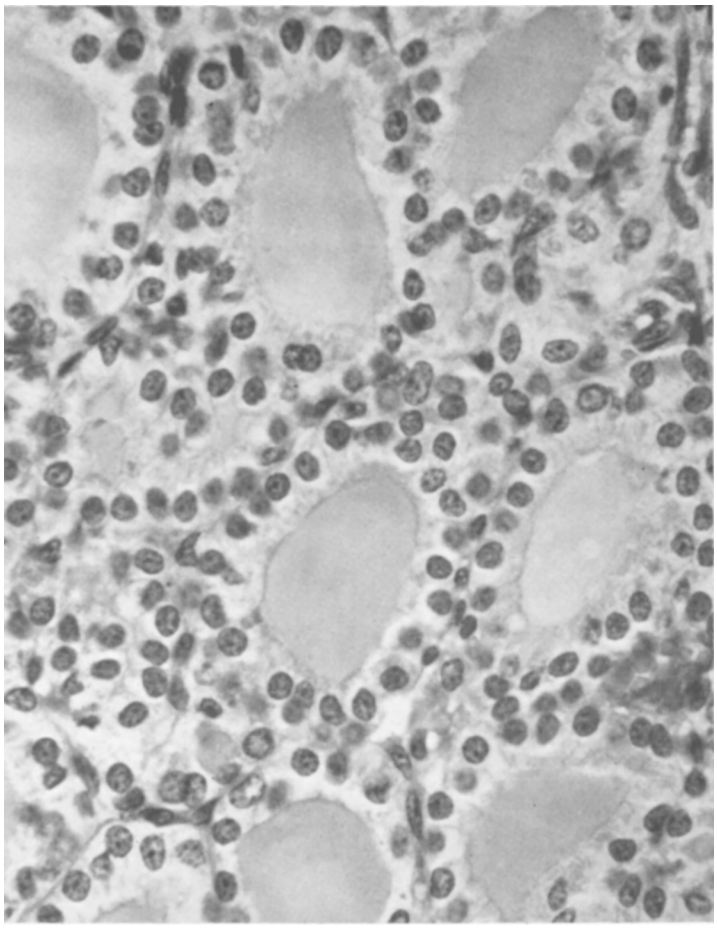

FIG. 2. Thyroid gland from control female Long-Evans rat of same age as in Fig. 1. Bouin fixation, hematoxylin and eosin. (×600.)

amino, 5-nitrothiazole on the thyroid gland or its production of thyroid hormone. Also, the reports that this compound interrupts egg laying(8,9) are consistent with the suggestion that 2-amino, 5-nitrothiazole might produce abnormalities in pituitary-thyroid function by reason of its antithyroid properties.

Summary. Rats fed 2-amino, 5-nitrothiazole had larger thyroid glands and a lower thyroidal accumulation of I^{131} than non-treated rats. It is suggested that the antithyroid properties of this compound might explain the inhibition of secretion of pituitary gonadotropin that has been reported following the administration of 2-amino, 5-nitrothiazole to fowl.

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