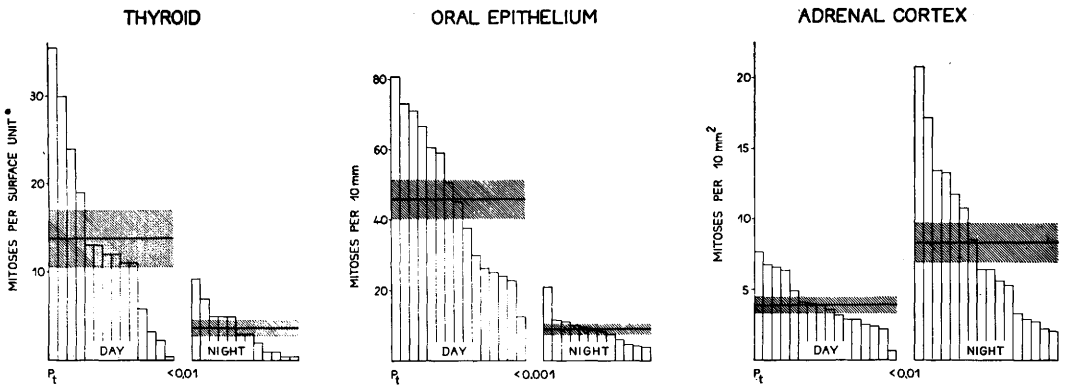

* Mitoses/150 oil-immersion fields.

FIG. 1. Mitotic activity at two times of day in tissues investigated. (Columns show data for individual rats; horizontal lines and shaded areas denote means ± 1 S.E.; day = 7:00-7:30; night = 21:00-21:30.)

adrenal constitutes a fundamental mechanism of physiologic daily periodicity, the phase-difference noted herein between its mitotic periodicity and the 24-hour spacing of other rhythms may call for a detailed analysis of adrenal mitotic activity (with more frequent sampling during the 24-hour period).

Summary. Daily periodicity characterizes mitotic activity in the adrenal, the thyroid and the oral epithelium of male Sherman albino rats, kept under standardized environmental circumstances. The daily times of "high" and "low" in number of mitoses are roughly synchronized for the thyroid and the oral epithelium, while the 24-hour periodicity in mitotic activity of the adrenal cortex shows a different timing.

1. Mühlemann, H. R., Zander, H. A., and Halberg, F., *J. Dent. Res.*, 1954, v33, 459.

2. Halberg, F., Zander, H. R., Houglum, M. W., and Mühlemann, H. R., *Am. J. Physiol.*, 1954, v177, 361.

3. Bullough, W. S., *Biol. Revs. Cambridge Phil. Soc.*, 1952, v27, 133.

4. Halberg, F., *Journal Lancet*, 1953, v73, 20.

5. Marthaler, T. M., *Oral Med. and Oral Path.*, in press.

6. Kaine, H. D., Seltzer, H. S., and Conn, J. W., *J. Lab. and Clin. Med.*, 1955, v45, 247.

7. Halberg, F., Barnum, C. P., and Vermund, H., *J. Clin. Endocrinol. and Metab.*, 1953, v13, 871.

Received October 7, 1955. P.S.E.B.M., 1955, v90.

Oxytocic Action of Rat Hypothalamus Extracts. (22068)

H. CROXATTO AND B. ZAMORANO.

Laboratorio de Fisiología del Instituto Pedagógico Universidad de Chile, Santiago.

Vogt(1) confirmed the presence of vasopressor, antidiuretic and oxytocic activities in extracts of dog hypothalamus. According to her results the hypothalamus differs from the posterior pituitary in that the relative oxytocic activity of the hypothalamus is much lower than the expected value, the P/O ratio (pressor activity/oxytocic activity) being 14.6. Inasmuch as Lawler and du Vigneaud (2) have shown that oxytocin-free vasopressin possesses an oxytocic activity corresponding to 5% of the pressor activity, Vogt(1) suggests that the oxytocic substance extracted from the hypothalamus may be pure vasopressin. This interpretation is of importance in view of the findings of Bargmann and Scharrer(3) on production of hormones of the pars nervosa of the hypophysis by the neuro-

secretory nuclei of the hypothalamus. These observations led Vogt(1) to deduce that either the oxytocin is chemically changed by the hypothalamus or this organ produces oxytocin and its nuclei the vasopressin.

Methods. The comparative effect of several crystalline proteolytic enzymes: pepsin, chymotrypsin, trypsin and carboxypeptidase, on extracts of hypothalamus and posterior pituitary of the same rat was studied in order to determine whether the oxytocic activity of the hypothalamus might be due to a substance similar to either oxytocin or vasopressin. Croxatto(4) has shown that the oxytocic activity is destroyed by chymotrypsin, but it is not affected by carboxypeptidase, pepsin and trypsin; on the other hand vasopressin is inactivated by chymotrypsin and also by crystalline trypsin. Lawler and du Vigneaud (2) using a highly purified preparation of vasopressin have confirmed the latter finding. Extracts were obtained from the anterior hypothalamic region of male and female adult rats, comprising a triangular area based on the posterior boundary of the optic tractus, from which portions of the tuber cinereum were definitely excluded. The small portion tested contained the supraopticus and paraventricularis nuclei. The tissue thus obtained from 2 or 3 rats was ground with micronized quartz in a mortar, the temperature maintained at 0°C. The ground tissue was suspended in 0.9% NaCl, in the proportion of one ml per hypothalamus. The clear supernatant fluid obtained on centrifuging at low temperature was divided in 2 equal portions, to one of which 2 to 3 mg of enzyme were added. Both samples were incubated at 37°C for one to 6 hours at pH 7.3, except that in the case of pepsin the pH was 2.5. After incubation the extract was neutralized and tested on rat uterus. Some of the incubation mixtures were brought to pH 2.5 and deproteinized by addition of 10 volumes of alcohol, centrifuged, the alcohol in the supernatant evaporated and the residue taken up in Ringer's solution. The posterior lobes of the hypophysis of the same animals were subjected to the same treatment as the hypothalamus.

TABLE I. Oxytocic Activity (mU) Anterior Hypothalamus Extracts.

Before incubation	After incubation: No enzyme	After incubation: Enzyme added		Hr
.75	.5	.7	Pepsin	1
	.5	.5		1
	.5	.75		5
1.-	1.0	1.0		5
.5	.5	.5		5
	1.5	.5	Chymotrypsin	1
	*1.5	1.5		1
	1.0	.5		1
	1.0	.1		2
	1.0	.1		2
.5	.4	0		6
	.2	0		6
	.5	.75	Trypsin	4
.5	.2	.2		6
	.2	.2		6
	.40	.5		6
	.2	.4		6
.5	.3	.3		6
.6	.5	.5	Carboxypept.	2
	.3	.3		4
	† .2	.4		4
	.3	.3		6

Extracts were incubated at pH 7.3 except in the case of pepsin the pH was 2.5; in (*) the pH was 3 and in (†) the pH was 8.2.

Results. The effect of pepsin, carboxypeptidase, trypsin and chymotrypsin on extracts of hypothalamus was the same as that observed for extracts of the posterior pituitary (Table I). Chymotrypsin was the only enzyme that caused a complete or almost complete loss of the oxytocic activity of hypothalamus and posterior pituitary extracts. No decrease in oxytocic activity of both extracts was produced by the other enzymes tested. In some experiments trypsin, carboxypeptidase and pepsin produced a slight increase of the oxytocic activity of both extracts. Incubation of the extracts at pH 7.3 with no addition of enzyme resulted in an appreciable loss of oxytocic activity by both extracts.

If one takes into consideration the specificity of the enzymes tested, our results would strongly support the hypothesis of the chemical identity of the substances from the hypothalamus and the posterior pituitary of rats. Contrary to the assumption of Vogt(1) regarding dog hypothalamus, the oxytocic activity of rat hypothalamus appears to correspond to that of a substance like oxytocin rather than one like vasopressin.

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.

1. Vogt, M., *Brit. J. Pharmacol.*, 1953, v8, 193.
2. Lawler, H., and du Vigneaud, V., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 114.
3. Bargmann, W., and Scharrer, E., *Amer. Scientist*, 1951, v39, 255.
4. Croxatto, H., *Rev. Med. y Aliment.*, 1942, v5, 259.

Received October 13, 1955. P.S.E.B.M., 1955, v90.

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

C. J. SHELLABARGER AND F. C. SCHATZLEIN. (Introduced by E. P. Cronkite.)
Division of Experimental Pathology, Medical Department, Brookhaven National Laboratory, Upton, L. I., N. Y.

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

* This work was supported by the U. S. Atomic Energy Commission.

† "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.