It was equally active by oral and subcutaneous routes. Doses of 0.1 mg/kg/day and greater interrupted the estrous cycles of young mature female rats. Treatment during cohabitation produced a loss in fecundity which was reversible after cessation of treatment. Pituitaries of chloramiphene-treated rats had approximately the same weight and gonadotrophin content as control rats. Chloramiphene did not inhibit exogenously administered gonadotrophin.

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Effect of Reserpine on Pituitary-Gonadal Axis.* (26055)

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Interference of tranquilizing agents, particularly chlorpromazine and other phenothiazine derivatives, with adrenal, thyroid and gonadal functions has been reported by several investigators(1-5). Most authors ascribe this interference to action of the drug on the hypothalamus. Since the hypotensive and tranquilizing effect of reserpine is presumed to result from its action on the diencephalon, and particularly on the hypothalamic region (6-7), reserpine might be expected to interfere with the normal functioning of the endocrine system.

Reserpine was reported to interfere with estrous cycles in mice(8), ovulation and menstruation in monkeys(9), prolactin secretion and discharge in rats, rabbits and women (10-12), oxytocin and prolactin secretion in lactating rats(13), and to effect recession of exophthalmus(14).

In this paper we wish to report on the effect of reserpine on the pituitary-gonadal axis.

Materials and methods. Reserpine stock solution was prepared according to the method described in Martindale's Extra Pharmacopoea 1959, and diluted to the required concentrations. Experiments were carried out on a total of 200 male and female infantile and adult albino rats and 10 male pigeons of the Columba strain. Vaginal smears and gonadotropin determinations were performed on 8 female patients.

The following aspects of the effect of reserpine on the pituitary-gonadal axis were studied: A. Vaginal pattern in infantile rats, adult rats and women; B. Ovarian and uterine development in infantile and adult rats; C. Testicular development in infantile male rats, adult male rats and adult male pigeons; D. Gonadotropin content in urine of menopausal women.

Results. A. Vaginal Pattern. 1. *Infantile female rats.* Twenty rats were injected subcutaneously with 0.05 mg/kg reserpine and another 20 rats with 0.2 mg/kg; 20 controls received the vehicle. In 10% of controls, vaginal opening was complete at age of 35 days, and in 50% at age of 44 days. After this date positive vaginal smears in control animals varied between 20-50%. At dose level of 0.05 mg/kg the stage of vaginal opening and incidence of positive smears closely resembled the pattern observed among the controls (see above). At dose level of 0.2 mg/kg a delay in vaginal opening was ob-

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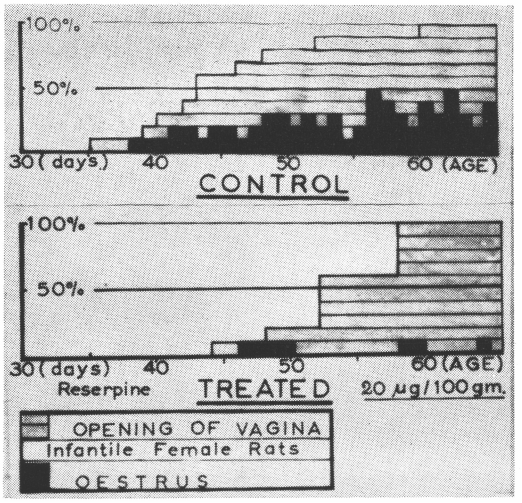

FIG. 1. Effect of reserpine on vaginal opening and estrus in infantile female rats during 40 days' treatment.

served. No opening occurred by the 35th day, there were only 10% openings on the 44th day and 50% on the 52nd day. Daily estrous incidence sank to 10% (Fig. 1).

2. *Adult female rats*: Ten rats which showed normal and regular estrous cycles of 4-5 days were injected subcutaneously with 0.2 mg/kg reserpine daily for 2 successive periods of 15 days each, at an interval of 25 days. Reserpine treatment resulted in a state of diestrus after 3-5 days of treatment, which continued for 3-5 days after withdrawal (Fig. 2).

3. *Women*: Ovarian activity of 3 female patients, 30-40 years old, who received daily reserpine treatment (5-15 mg/d) over a period of 20-60 days, was examined by vaginal smears. They showed successively decreasing estrogen activity throughout treatment.

B. Ovarian and uterine development. 1. *Infantile female rats*: Two groups of 10 rats received subcutaneously daily 0.1 mg/kg and 0.25 mg/kg body weight reserpine, respectively, for 30 days; a third group of 10 rats served as control. At the end of this treatment all animals were sacrificed and their ovaries and uteri were dissected and weighed, showing ovarian weights of 30-36 mg in the first group (0.1 mg/kg reserpine daily) and 8-13 mg in the second group (0.25 mg/kg), as compared with 42-50 mg in the controls. Uterine development was similarly affected, the respective figures being 185-220 mg for the first group, 85-110 mg for the second, as against 300-420 mg in the controls.

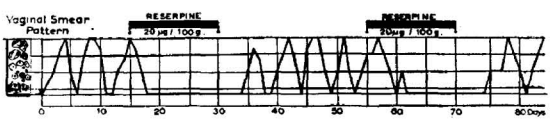

FIG. 2. Vaginal pattern of adult normally cycling female rat during 2 successive treatments with reserpine.

2. *Adult female rats*: Two groups of 10 rats received subcutaneously daily 0.1 mg/kg and 0.2 mg/kg reserpine, respectively, for a period of 40 days; a third group of 10 rats served as control. At the end of this period the animals were sacrificed and ovaries and uteri examined morphologically and histologically. While reserpine caused only slight losses in weights of uterus and ovary, it provoked pronounced morphological and histological changes. No fully mature follicles were found, corpora lutea were highly developed and uteri were atrophic as in lactating rats, suggesting intensified prolactin secretion from the pituitary.

C. Testicular development. 1. *Infantile male rats*: Four groups of 10 rats each were daily injected subcutaneously with 0.01, 0.05, 0.1 and 0.2 mg/kg reserpine, respectively, for 30 days. A fifth group of 10 rats served as control. All animals were examined for testicular descent. While doses of 0.01 mg/kg reserpine daily did not delay testicular descent, an obvious delay was seen at doses from 0.05 mg/kg upwards. The higher the dose, the longer the delay.

2. *Adult male rats*: Four groups of 5 adult male rats each, weighing 200 $\pm$ 15 g, received subcutaneous injections of 0.05, 0.1, 0.25 and 0.5 mg/kg reserpine, respectively, for a period of 15-40 days. Their testes, seminal vesicles and prostates were examined. Low doses of reserpine of 0.05 mg/kg were found to have no effect on morphology or histology of testes, seminal vesicles and prostates. In some, but not all, animals, higher doses caused severe atrophy of the seminal vesicle and prostate and regressive changes in the testes, as seen after hypophysectomy (Fig. 3).

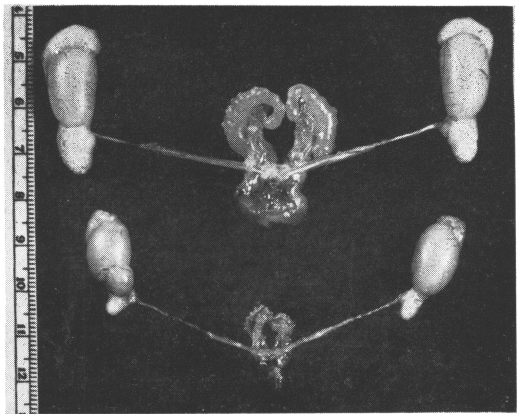

FIG. 3. Accessory sex organs of normal adult rat (above) and reserpine treated adult rat (below).

3. *Adult male pigeons*: Five male pigeons weighing between 250-300 g were given 0.2 mg/kg reserpine. This caused a very severe atrophy of the testes (loss of weight 50-80%). Histological sections of the testes showed impaired spermatogenesis, arrested at the stage of spermatids or mitotic secondary spermatocytes. In rare cases spermatozoa appeared in small numbers and Leydig cells ranged between 3-15 per triangle with an average of 7, while in controls they ranged between 6-20 with an average of 12 (Fig. 4).

While the atrophy of the testes could be the result of diminished secretion of FSH, LH, it could also be due to increased prolactin secretion, which has an antigonadal effect, or to both.

D. Gonadotropin excretion in urine of menopausal women. The gonadotropin content of the urine of 5 female patients, aged between 45-55 years, who were to receive reserpine treatment, was established. Two had 100 RU/L and one 50 RU/L. The remaining 2 had less than 50 RU/L. They received 5-15 mg reserpine daily over 40 days. Their gonadotropin excretion decreased during treatment to 25 RU/L and remained low for 3 months.

Discussion. Whereas reserpine had a relative antigonadotropic effect on male, female, infantile and adult rats, it brought about complete aspermatogenesis and up to 80% atrophy of the testes in pigeons. The severity of the effect of reserpine in pigeons might be due to simultaneous depression of FSH and LH secretion and increased prolactin secretion and discharge, since the latter has an antigonadal effect in pigeons(15,16).

Although administration of reserpine resulted in loss of weight due to decreased food intake, this could not be the cause of the disturbance in the pituitary-gonadal axis, as partial starvation for 3 months did not bring about any of the changes observed.

Other investigators(17) found that ovulation and pregnancy in rats are disturbed by reserpine treatment. Electroencephalographic examination showed that administration of reserpine or chlorpromazine to adult rats before 2 p. m. on day of proestrus inhibited the typical ovulation waves and ovulation (18).

We have reported our hypothesis for the mechanism of increased prolactin production and secretion for chlorpromazine(2,5). This probably also holds true for reserpine. Following suppression of the hypothalamus, a secondary impairment of pituitary and peripheral glands will occur. With regard to part of the pituitary tropins, this suppression becomes balanced according to the push and pull principle, which applies readily to the tropins producing peripheral hormones. As prolactin (LTH) produces milk only, an overshoot of this tropin may occur. This would classify it as an unbalanced tropin, as distinct from other, balanced tropins, which are held in abeyance by a feed-back mechanism of their own peripheral hormone production.

Summary. Reserpine at a dose level of 0.2 mg/kg delayed vaginal opening in infantile female rats, and postponed estrus in normally cycling female rats. In women, vaginal smears likewise indicated decreased estrogen

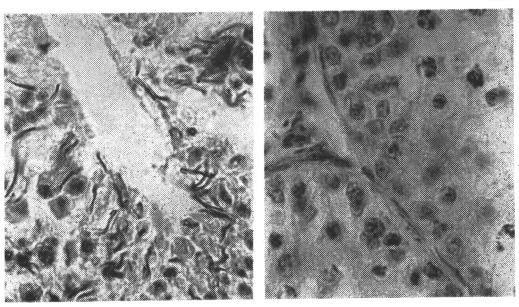

FIG. 4. Histological section of testes (380 ×). Left, normal male pigeon; right, reserpine treated male pigeon. Note absence of sperms in latter.

production throughout treatment. Histological sections of the ovaries of adult rats treated with reserpine showed absence of mature follicles (control 5-8), and presence of corpora lutea, similar to those seen during lactation. Sections of uteri showed the same pattern as in lactating rats. Reserpine delayed testicular descent in young infantile rats. In mature male rats, high doses resulted in regressive atrophy of testes, seminal vesicles and prostates. Testes of pigeons treated with reserpine weighed 20-50% of the weight of controls. Histological sections showed arrest of spermatogenesis at the state of spermatids or mitotic secondary spermatocytes. Only in rare cases were spermatozoa found in relatively small numbers. The gonadotropin level in menopausal women who received reserpine treatment in doses as high as 5-15 mg daily, decreased from 100 RU/L to 25 RU/L. It is concluded that reserpine interferes with the pituitary-gonadal axis by suppression of gonadotropin secretion from the pituitary, apparently as a result of its direct action on the hypothalamus.

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