Failure to Detect Estrogenic or Gonadotrophic Activity in Ergotoxine.* (22977)

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Ergotoxine produces manifestations of increased systemic estrogen in early pregnant and pseudopregnant rats(1). These include: termination of pregnancy and pseudopregnancy with the appearance of estrous type vaginal smears within 96 hrs; and the suppression of the decidual cell reaction(2). The injection of supplemental progesterone overrides or reverses the ergotoxine action(2,3). The ergotoxine effect may be due to direct estrogenic activity, indirect estrogenic by gonadotrophic (FSH) action, or by interference with progesterone utilization which would reflect itself as an increased estrogen status.

In attempting to elicit information on site and mechanism of the alkaloid drug action, tests for estrogenic and gonadotrophic activity were carried out in rats treated with ergotoxine. This is a report of the assays.

Methods. Assays for estrogenic activity were carried out on 40 spayed adult female rats (bd wt 180 $\pm$ 6.7 g), and 44 immature rats (*22/23* days old; 47.0 $\pm$ 7.2 g). The immatures also serve for assay of gonadotrophic activity(4).

Results. 30 adult females were spayed and 5 days later injected with 0.5 mg ergotoxine methanesulphonate† (0.1 ml 95% ethanol); 10 females were primed with 1 γ stilbesterol 10 days after ovariectomy, and 5 days later injected with 0.5 mg ergotoxine twice daily x 3 days. Vaginal smears were examined daily during and for 7 days after treatment. No evidence of estrogenic activity was seen in smears, nor was there any observable change in the uterine status to indicate modification of hormonal deprivation.

44, *22/23*-day-old immatures were divided into 28 ergotoxine, and 16 alcohol treated. Experimental animals received 1.5 mg ergotoxine in 0.2 ml 95% ethanol; controls received only alcohol. 72 hrs later uteri were removed, trimmed and weighed. Uteri of ergotoxine-treated rats weighed 66.5 $\pm$ 17.02 mg/100 g bd wt: control uteri were 66.1 $\pm$ 15.1 mg/100 g bd wt. There is no significant difference ($t = 0.08$; $P > 0.9$).

The weight response and histology of the ovaries of the immature rats (II) was used as an index of gonadotrophic activity. Ovaries of ergotoxine-treated rats weighed 47.8 $\pm$ 8.8 mg/100 g bd wt: and of control rats 50.6 $\pm$ 8.9 mg/100 g bd wt. There is no significant difference ($t = 1.010$; $0.4 > P > 0.3$). There is no evidence of gonadotrophic activity from ergotoxine. Microscopic study of H & E preparations revealed no structural differences between ovaries of ergotoxine-treated rats and controls.

Conclusions. Amounts of ergotoxine methanesulphonate which effectively terminate pregnancy, pseudopregnancy and deciduoma formation(1-3), failed to show estrogenic or gonadotrophic activity as tested in adult spayed, and immature intact rats. The termination of pregnancy and pseudopregnancy with a concomitant appearance of estrous configuration in the vaginal smears is therefore not due to any inherent estrogenic or gonadotrophic activity of the ergotoxine.

Summary. Ergotoxine methanesulphonate was tested on adult spayed, and immature intact rats, for estrogenic and gonadotrophic activity. None was found. The action of ergotoxine with respect to terminating early pregnancy and pseudopregnancy cannot be explained on the basis of estrogenic activity in ergotoxine.

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† Ergotoxine methanesulphonate was an 1:1:1 mixture of ergocornine, ergocristine, and ergokryptine methane sulphonate, made available through the courtesy of Dr. A. Cerletti, Sandoz.

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Comparison of Atrophy and Glycogen Storage in Some Muscles After Castration and Denervation.* (22978)

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Castration in adult male rats causes atrophy and loss of glycogen in perineal muscles (including levator ani) and testosterone restores the muscles to normal(1,2). Denervation of skeletal muscle (gastrocnemius) also causes loss of glycogen, coincident with atrophy(3,4). The present study is concerned with a comparison of atrophic and glycogen changes which follow castration and denervation in the male hormone sensitive levator ani and cremaster muscles. The effect of some hormones on glycogen deposition in denervated muscles is also presented.

Methods. Adult male rats of matched body weights were employed, except where indicated, and all operations were performed using ether anesthesia. The genito-femoral nerve to the cremaster was evulsed just posterior to the ilio-lumbar vessels. The nerves forming the pudendal plexus on each side of animal were destroyed to denervate the perineal muscles. A section of the posterior division of the femoral nerve was removed to denervate the rectus femoris muscle. Wherever it was obviously feasible, the right muscle was denervated and the left one used as control, otherwise separate groups of rats were used. In experiments with hormone treatment, testosterone propionate (1 mg doses) and cortisone acetate (2 mg doses) were administered daily for 3 days prior to autopsy.† A 24-hour fast preceded the autopsies and muscles were removed from the anesthetized (nembutal) rats. Only the levator ani of the perineal muscle group was used because of its discrete nature. Varying periods of time elapsed after the operations and before autopsy. Weighing and glycogen determinations were made as previously described(5).

Results. The data in Table I show weight changes in the rectus femoris, cremaster and levator ani muscles following castration and denervation. Fifteen days after denervating the right rectus femoris, the muscle weighed 48% less than the control. Previous observations have shown no appreciable weight loss in this muscle after castration and no data are recorded.

The weight of the levator ani 15 days after either denervation or castration was less than the controls but denervation caused a greater loss in weight. In contrast, there was no significant weight loss in the cremaster 15 days after either denervation or castration.

On the assumption that a time factor might be involved in the latter instance, another group of rats was castrated and autopsied 32 days later. A significant weight loss in the cremaster now occurred. For the denervation experiment, young male rats (120-140 g body weight) were employed and 42 days elapsed after denervation of the right cremaster before autopsy. In addition, reference controls were obtained on day of operation by weighing cremaster muscles from a similar group of young males. The weights of the cremaster muscle 42 days after denervation were significantly less than the paired controls. How-

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