inhibiting hemagglutination by H-1 virus since it is active in concentrations as low as 0.006 μg/ml.

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A Study of Estrogen-Sensitive Hypothalamic Centers Using a Technique for Rapid Application and Removal of Estradiol* (33000)

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Harris *et al.* (1) reported eliciting estrous behavior in spayed female cats by placing stilbesterol implants in the hypothalamus. Various workers since have investigated the effects of sex steroids on estrogen sensitive centers in the cat (2) rat (3,4) and rabbit (5). From the work of Lisk (3,4) it now seems quite evident that in the rat there are two estrogen-sensitive centers in the hypothalamus. One, located anteriorly and just dorsal to the optic chiasm, is concerned with initiation of sex behavior. The second center, located in the median eminence region of the posterior hypothalamus is apparently concerned with the release of gonadotropins from the pituitary. The female rabbit appears to differ from the above named species in that estrous behavior has been reported when estrogen was implanted into the ventromedial nucleus of the hypothalamus (5).

Materials and Methods. Previous studies have been carried out in groups of animals with permanently implanted tubes. We have devised a simple method which makes it possible to remove estrogen implants. A 6-mm length of 22-gauge stainless steel tubing is cut and polished. This short piece of tubing, which we call a "guide barrel" is stereotaxically placed in the calvarium and firmly affixed by means of dental cement. A piece of 27-gauge tubing attached to the stereotaxic chuck serves as a carrier for the "guide barrel." After the placement holes have been drilled, the "guide barrel" is slipped down the 27-gauge carrier tube into the brain, leaving a 3-mm projection above the surface of the calvarium, the 3-mm line having previously been measured and marked. Dental cement is then applied, and once hardened, holds the "guide barrel" in a rigidly fixed position. Following placement of the "guide barrel," a short piece of 27-gauge tubing, bent at one end for easy removal with forceps, is placed in the 22-gauge tubing and serves as a plug (Fig. 1).

The estrogen-tipped tubes were prepared for implantation in the following way. Estradiol was brought to the melting point and by means of polyethylene tubing with a hypodermic syringe attached to one end of the steel tubing, a negative pressure was created to

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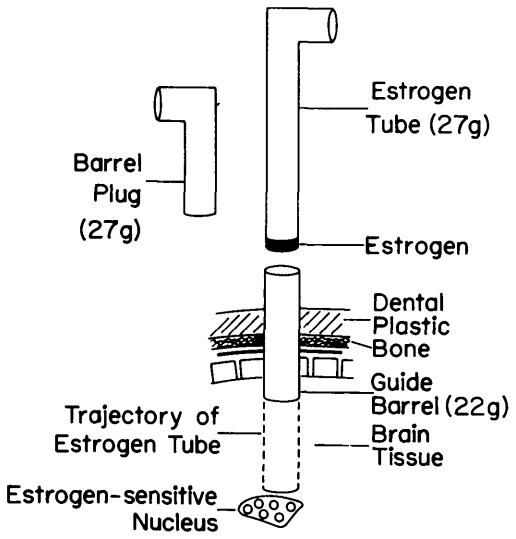

FIG. 1. The 22-gauge "guide barrel" was positioned stereotaxically and affixed to the calvarium by dental cement. A trajectory of its lumen would extend to the estrogen sensitive nucleus. The distance from the top of the guide barrel to the surface of the nucleus was calculated, and a sharp bend was placed in the estrogen tube.

help draw the molten hormone up into the lumen of the 27-gauge tube. The amount of steroid taken into the tube is not crucial since only the open end is in contact with brain tissue, and in comparable experiments dissolution of hormone in the brain was not measurable by weight loss of hormone from the tube (4). We scraped all the steroid from the outer surface of the estrogen tube while viewing through a dissecting microscope. Finally the tube was sponged off with ether–alcohol prior to placement in the brain. With the rat stereotaxic atlas (6) the desired length was determined for the estrogen tipped tube and a sharp right angle bend was made in the tubing opposite the estrogen tip. The lumen of the estrogen-filled tube measured approximately 200 μ. Dissolution of hormone in the brain is not measurable by weight loss of hormone from the tube (4).

The efficacy of this technique was demonstrated in experiments on four ovariectomized and nine intact female rats implanted by the method described above. Blank tubes lowered through the "guide barrels" had no physiological effect on estrous behavior.

Results and Discussion. Although it has been shown (4) that 27-gauge estradiol tubes implanted in the preoptic–suprachiasmatic region of the castrated female rat produced lordosis and mating after 3–5 day latency, all four of our ovariectomized females exhibited lordosis and mated within 24–36 hours after implantation. At the time of estrogen placement, the unanesthetized rat was held, and the tube was slipped down the "guide barrel." This technique eliminates the effect of a surgical procedure at the time of estrogen application and may account for our shorter latency period.

In five normally cycling intact rats estrogen-tipped tubes were placed in the arcuate region of the posterior hypothalamus for 7 days. All rats showed constant vaginal diestrus. Following the removal of the estrogen tubes normal vaginal cycles returned in 16–37 days. Estradiol implants in the basal tuberal–medial eminence region produces ovarian atrophy in the rat (3) and rabbit (7). Electrolytic destruction of this region produces the same phenomenon (8). The delay in vaginal cycling seen following removal of the estrogen tube from the posterior hypothalamus is apparently related to the reproductive tract atrophy observed by Lisk (3) and Davidson and Sawyer (7). The technique used in this study permitted us to remove the estrogen tube and observe that the functional atrophy was reversible.

In four intact females, estrogen tubes were placed in the anterior hypothalamus and left in for 30 days to determine the effect on vaginal cycles. Two animals cycled within the normal range of 5–7 days, but two did not cycle until the twelfth and twenty-sixth day, respectively. Histological examination at the end of the experiment showed that the tube locations in the latter animals were just above the most caudal part of the otpic chiasm, thus the interference with normal cycling might be related to diffusion into the arcuate area, which lies a little less than 1 mm caudal to the tip of the estrogen tubes. Regardless of the interference with normal cycling in two animals in the group, the effect was transitory. After the animals had all cycled normally for 3 weeks, the tubes were removed from the

anterior hypothalamus and lowered via the posterior "guide barrel" into the arcuate nucleus area. All four were diestrus within 2 days and remained so until sacrificed 1 month later.

Summary. The effect of estrogen-tipped steel tubes has previously been studied with permanent implant methods. An improved technique makes it possible to place and remove 27-gauge estrogen-tipped tubes through a short 22-gauge "guide barrel" permanently implanted in the calvarium. The "guide barrel" is implanted and the animal allowed to recover from the effects of surgery prior to placement of the estrogen tube. Three experiments are described demonstrating the value of this technique: (i) Castrated female rats with estrogen tubes placed in the preoptic–suprachiasmatic region of the anterior hypothalamus exhibited behavioral lordosis and mating in less time than that previously described for rats with permanent tube implants. (ii) Estrogen tubes placed in the estrogen-sensitive center of the posterior hypothalamus of intact, normally cycling female rats for 7 days suppressed the return of normal vaginal cycles for 16–37 days after the tubes were removed. In previous reports with permanently implanted tubes it was not possible to examine the delay in return to normal vaginal cycling following estrogen application to this hypothalamic center influencing gonadotropin release. (iii) Intact female rats with estrogen tubes placed in the anterior estrogen-sensitive center of the hypothalamus showed only temporary interference with normal vaginal cycling.

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Indirect Sodium-Retaining Action of Oxytocin on Dog Kidney (33001)

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The effect of oxytocin on renal salt and water excretion was found in past investigations to depend on the animal species studied, the dose applied, and the basal rate of urine flow. In the rat, injections of 10–100 mU of oxytocin per animal consistently increased renal sodium, chloride, and water excretion (1, 6–9). In the dog, oxytocin injected intravenously in dosage of about 10 mU/kg of body weight induced natriuresis only at low rates of urine flow (2–4,7). Least consistent results were obtained in human studies; some workers were unable to demonstrate any effect of oxytocin on renal water and electrolyte excretion (5,13), while others reported inconstant antidiuresis and antinatriuresis (10). It was usually admitted that in various species oxytocin possesses about 1/100 of antidiuretic potency of vasopressin (3,13).

Brooks and Pickford observed that when oxytocin was injected into the carotid artery

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