THE administration of progestational compounds, both natural and synthetic, has proved to be an effective method of controlling estrous cycles. It seems clear that progestins suppress ovulation by reducing the secretion of pituitary gonadotropins. The events leading to ovulation can be divided into the following three steps: (1) an ovulatory signal originates in brain tissue outside the median eminence, (2) the signal is relayed to the median eminence where it causes hypophysiotrophic hormones to be released into the pituitary circulation, and (3) the hypophysiotrophic hormones, upon reaching the pituitary, cause the release of gonadotropin (Malven, 1970). Progestins may act to prevent ovulation at any or all points along this sequence of events. One method of experimental investigation has been to insert an artificial ovulatory stimulus in progestin-treated animals between steps 1 and 2 or between steps 2 and 3. If progestin treatment still blocks ovulation in response to the artificial stimulus, its site of action must be distal to the site at which the stimulus was inserted. Using this approach, Spies et al. (1969) suggested that progesterone and chlormadinone prevented ovulation in rabbits at a site distal to the median eminence. Stevens et al. (1970) confirmed their results with progesterone in rats.

The site(s) of progestin action also has been studied by intracranial implantation. Docke, Dorner and Voigt (1968) prevented ovulation in rats by either intrahypothalamic or intrapituitary implantation of chlormadinone. Smith, Weick and Davidson (1969) reported inhibition of ovulation by intrahypothalamic implants of progesterone. In all these implantation experiments, designation of a hypothalamic site of progestin action is complicated by possible drainage of material from intrahypothalamic implants via the hypophysial portal blood vessels to the anterior pituitary (Palka, Ramirez and Sawyer, 1966).

The present experiments used intracranial implants of the synthetic progestin, medroxyprogesterone acetate, in an attempt to define sites of action. Pasteels and Ectors (1968) reported that intrahypothalamic implantation of this compound delayed ovulation in rats. The present studies were conducted in guinea pigs, the species in which Malven (1971) showed that the nonsteroidal inhibitor, methalibure, acted on hypothalamic rather than pituitary tissue.

Materials and Methods

Adult female guinea pigs weighing 550 to 900 g were housed under a controlled lighting schedule of 14 hr. light per day. Estrous cycles were followed by daily examination of the vaginal closure membrane. Day 0 of the cycle was the first day of a series of days in which the membrane was absent and the vagina was open. Estrous cycle lengths of 13 to 21 days were observed prior to treatment.

Medroxyprogesterone acetate (MAP) was prepared for intracranial implantation in the lumen of 24 gauge stainless steel tubing. Small amounts of MAP were melted, and one end of the tubing was dipped into the liquid steroid. MAP was drawn up into the lumen by capillary action. After cooling overnight, each MAP-filled tube was scraped and then wiped with lens paper soaked in ether to remove any steroid on the outside of the tubing. Each tube was examined under a dissecting microscope to insure that very little steroid protruded from the lumen of the tube. The amount of material contained initially in the implant tubes was not measured because the appearance of the implants changed very little during 8 days of intracranial implantation.

On day 11 of the estrous cycle, each ex-
Experimental animal was anesthetized, placed in a stereotaxic instrument, and implanted with a single unilateral implant tube as described by Malven (1971). Most animals received MAP-filled implants but some animals, also reported by Malven (1971), received implants of empty tubing. Seven animals were implanted subcutaneously with large pellets of previously melted MAP weighing 38 to 112 milligrams.

Eight days after implantation (day 19) all animals were killed, and the heads were perfused with saline and 10% formalin. The exact location of each intrapituitary and intrahypothalamic implant was determined as previously described (Malven, 1971). Ovarian and vaginal histology was also examined as in the previous study.

Results and Discussion

Table 1 summarizes the various types of implants used and the fraction of animals which lacked new corpora lutea (CL) when examined on day 19. All eight animals with empty intrahypothalamic implants had ovulated and their ovaries contained young CL. The vaginal epithelium had returned to a relatively thin diestrous condition. All seven animals with subcutaneous progestin pellets had not yet ovulated by day 19 even though their CL from the previous ovulation had greatly degenerated. In all seven animals the vaginal epithelium was mucified while in five the vagina was still closed on day 19. In four of the seven cases, the unruptured ovarian follicles had undergone a partial luteinization characterized by hypertrophy of the granulosal layer.

Unilateral implantation of MAP within the anterior pituitary prevented ovulation in seven out of 10 animals. The vagina had opened by day 19 in all but one of these animals. In this one case, the old CL from the previous ovulation had not completely regressed. In five of the seven animals in which ovulation was inhibited, the unruptured follicles were partially luteinized. The vaginal epithelium was mucified in all but two of the cases of ovulation inhibition. The partial follicular luteinization and the vaginal mucification in some of the animals in which ovulation was prevented contrasts somewhat with the results of intrahypothalamic implantation of methallibure (Malven, 1971). Whenever methallibure prevented ovulation, the vaginal epithelium was fully cornified and unruptured follicles were heavily luteinized in every animal. Apparently intracranial MAP implantation tends to partially inhibit the release of pituitary hormones responsible for follicular luteinization and estrogen secretion, whereas methallibure implantation did not. On the other hand, vaginal mucification is also indicative of the combined effects of progestin and estrogen (Allen, Hisaw and Gardner, 1939), and some MAP from the intracranial implants may enter the systemic circulation, reach the vaginal epithelium, and cause the mucification as it apparently did in animals with subcutaneous implants.

These experiments did not include any control implantations into the anterior pituitary. However, in five animals the intrapituitary implants of medroxyprogesterone were inserted through the pituitary, and upon necropsy they were found to protrude from the ventral surface of the gland. These five animals all ovulated and provided an excellent control for the surgical trauma of intracranial implantation and for any destruction of pituitary tissue or pituitary vascularity.

MAP implantation into the arcuate nucleus of the hypothalamus prevented ovulation in five out of 10 animals. In two of these cases of ovulation inhibition, the old CL were not completely regressed. It was impossible to determine how much, if any, these CL contributed to the inhibition of ovulation. In both cases of incomplete CL regression the luminal layers of the vaginal epithelium were mucified and in one of these animals the vagina was still closed. The old CL were completely regressed in the three other animals lacking new CL, and in two of them the vagina was still closed and ovarian follicles were partially luteinized. In three animals MAP implants were intended for the arcuate
nucleus but protruded from its ventral surface and were outside the brain. Since all three animals ovulated, they served as controls in addition to the eight animals with empty intrahypothalamic implants.

Unilateral MAP implants in hypothalamic sites other than the arcuate nucleus did not prevent ovulation in any of 12 animals. However, all sites were not adequately investigated, and there may exist other hypothalamic sites at which MAP may act to inhibit ovulation. The following were the number of animals with ineffective MAP implants located in each of the hypothalamic areas: four in ventromedial nucleus, three in anterior hypothalamic area, two in posterior hypothalamic area, and one each in the lateral hypothalamic area, preoptic area, and optic chiasma.

Discussion

The present results suggest that progestins can act directly on the anterior pituitary to inhibit ovulation. Such an action is consistent with the results of Spies et al. (1969) and Stevens et al. (1970) in rabbits and rats. An action on the pituitary could involve either the direct inhibition of gonadotrophin release or the inhibition of the action of some hypothalamic releasing factor on the pituitary. The effectiveness of intrapituitary implants of medroxyprogesterone acetate in guinea pigs also agrees with the effects of chlormadinone implants in rats (Docke et al., 1968).

The partial inhibition of ovulation by MAP implanted in the arcuate nucleus is difficult to interpret. Any substance implanted in this region in the rat is readily transported to the pituitary gland (Palka et al., 1966). It is impossible to determine if the implanted MAP was so transported in the guinea pigs of the present experiment. Smith et al. (1969) and Docke et al. (1968) also noted inhibition of ovulation after progestin implants in this region. However, Docke et al. (1968) reported that electrical stimulation of the preoptic area could overcome the inhibition caused by intrahypothalamic implants but not that caused by intrapituitary implants. It seems clear that progestins do inhibit the anterior pituitary gland directly, but an action on those hypothalamic areas regulating the anterior pituitary is also possible.

Summary

Medroxyprogesterone acetate, contained in the lumen of stainless steel tubing, was implanted intracranially in female guinea pigs on day 11 of the estrous cycle. Ovulation had occurred by day 19 in all controls implanted with empty tubing. Ovulation was inhibited by MAP implantation in 70% and 50%, respectively, of the animals with implants in the anterior pituitary or in the arcuate nucleus of the hypothalamus. MAP implants in other hypothalamic areas did not inhibit ovulation. MAP implants which passed through either the anterior pituitary or the arcuate nucleus and protruded from their ventral surfaces never inhibited ovulation. The present results indicate that this progestin can act directly on anterior pituitary cells to inhibit ovulation, whereas the nonsteroidal compound methallibure acted on neural structures to inhibit ovulation. MAP also may act on neural tissue of the arcuate nucleus, but the present experimental approach cannot prove or disprove such an action.

Literature Cited


