slope of the regression lines varied from experiment to experiment which may reflect differences in the average speed of these cell populations.

It has been reported that preincubation of neutrophils in a chemotactic medium renders neutrophils unresponsive to further chemotactic stimulation, even if the cells have been washed extensively. This phenomenon has been called 'deactivation'[13]. We find that neutrophils, which have been exposed to S-CAT 1.5.1. and washed, show normal random locomotion. They behave as if S-CAT 1.5.1. were still present in the cell compartment. The threshold for a chemotactic response of such cells is shifted to a higher concentration of S-CAT 1.5.1. and approximates the cytotaxin concentration used for preincubating the cells. The plateau of the response is lowered (figure, B). This may be considered as a tachyphylactic state in leucocyte chemotaxis. It may reflect a failure to readapt efficiently to an environment with low stimulus intensity. Adaptation, e.g. in visual perception[14] or in chemotaxis of slime moulds[15], is a quite complex phenomenon. Its role in leucocyte chemotaxis remains to be further evaluated.

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The influence of long term estrogen treatment on plasma prolactin levels induced by ether anesthesia in ovariectomized rats[1]

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Summary. 2 methods of continuous estrogen delivery, polyestradiol phosphate injection and implantation of Silastic capsules of estradiol-17 β, in ovariectomized rats induced increases in plasma prolactin in the afternoon (15.00–17.00) beginning at 1 week and continuing for 4–8 weeks. In addition these methods of estrogen treatment potentiated the ether-induced increase in plasma prolactin in the morning (9.00–11.00) beginning on week 2 and continuing for 3–8 weeks. These results indicate that estrogen activates the mechanisms that cause an afternoon surge in prolactin before potentiating a morning elevation induced by ether anesthesia.

Repeated injections of large amounts of estrogen for several days increases the prolactin content in the rat pituitary[2,3], as well as in the serum[4–8]. Estrogen may promote the relase of prolactin into blood by affecting hypothalamic neural mechanisms[9,10]. In contrast to the chronic stimulatory effects of estrogen, several types of stressors induce acute elevations in prolactin secretion. The most widely investigated response is that to ether inhalation. Ether has been shown to increase plasma prolactin in ovariectomized rats[11] and the response is exaggerated in the ovariectomized animal treated with estrogen for 1–3 weeks[12]. The purpose of the present study was to elucidate the effects of long-term estrogen treatment on the ether induced rise of prolactin in the morning when the levels are low, and in the afternoon when the levels are elevated.

Materials and methods. 30 mature, female Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Michigan) weighing 200–250 g were randomly divided into 6 groups. After 7 days of acclimation to lighting conditions (lights on from 6.00 to 20.00 h) all the rats were bilaterally ovariectomized. 1 group was injected with 0.5 mg polyestradiol phosphate (PEP; 1.0 mg Estradurin®, Ayerst Laboratories) s.c., and 4 groups received s.c. Silastic implants containing 12.5 or 25 mg crystalline estradiol-17 β at the time of ovariectomy. The implants were similar to those used by Legan et al.[14]. 4 sizes of Silastic tubing were utilized: Dow Corning No. 602–265 1.57 mm i.d., 2.41 mm o.d. 14 and 24 mm in length and Dow Corning No. 602–285 1.57 mm i.d., 3.18 mm o.d. also 14 and 24 mm in length. A 2 mm wooden plug was inserted into 1 end of the Silastic tubing and crystalline estradiol-17 β was packed into the open end of the tubing. Another 2 mm plug was inserted into the open end, and both ends were coated with Silastic Medical Adhesive Silicone Type A (No. 890 Dow Corning). The implants were incubated for 30 min in distilled water at 20 °C and wiped with ethanol prior to insertion into a s.c. pocket in the nape of the neck. Vaginal smears were obtained daily to monitor the effects of the estrogen treatments.

Beginning 1 week after ovariectomy and estrogen treatment and continuing once weekly for 8 weeks, a 2 ml blood sample was obtained from the orbital venous plexus using heparinized capillary tubes after 5 min of continuous ether anesthesia in the morning (9.00–11.00 a.m.) and again in the afternoon (15.00–17.00 p.m.). Animals were anesthesized by an initial exposure to ether vapor in a large container, followed by maintenance with a nose cone. The samples were centrifuged and the plasma was collected and stored at –20 °C until assayed.

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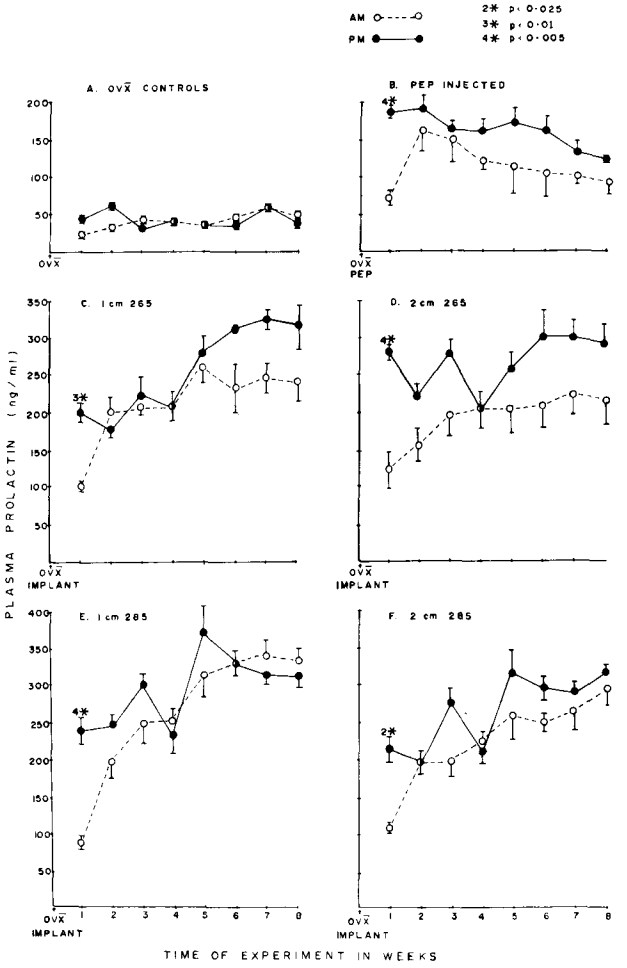
Plasma samples were assayed by double antibody radioimmunoassay [15] at 2 dilutions in duplicate. Rat Prolactin NIAMDD-RP-1 (11 IU/mg) supplied through the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism and Digestive Diseases was used as the standard.

One way analysis of variance[16] was used to assess statistical significance between the untreated ovariectomized and estrogen-treated groups, and between the a.m. versus p.m. values at each time period. Statistical analysis was used to compare prolactin levels between weeks within each experimental group.

Results. Ovariectomized, non-estrogenized rats exhibited no increase in plasma prolactin levels in the afternoon as compared to morning values. However, there was a tendency for the morning plasma prolactin levels to increase over the duration of the experiment.



Plasma prolactin levels in the morning (a.m., 9.00–11.00) and afternoon (p.m., 15.00–17.00) in ovariectomized and ovariectomized estrogen-treated rats bled from the orbital venous plexus following 5 min of ether inhalation. All values represent means ± SEM for 5 animals per group. *A* Ovariectomized controls. *B* Ovariectomized rats injected once s.c. with 0.5 mg polyestradiol phosphate (PEP). *C* Ovariectomized rats with a s.c. Silastic (Dow Corning No. 602–265, i. d. 1.57 mm, o.d. 2.41 mm, 1 cm in length) implant of estradiol-17 β. *D* Ovariectomized rats with a s.c. Silastic (Dow Corning No. 602–265, i.d. 1.57 mm, o.d. 2.41 mm, 2 cm in length) implant of estradiol-17 β. *E* Ovariectomized rats with a s.c. Silastic (Dow Corning No. 602–285, i.d. 1.57 mm, o.d. 3.18 mm, 1 cm in length) implant of estradiol-17 β. *F* Ovariectomized rats with a s.c. Silastic (Dow Corning No. 602–285, i. d. 1.57 mm, o.d. 3.18 mm, 2 cm in length) implant of estradiol-17 β.

In contrast, animals treated with polyestradiol phosphate (PEP) demonstrated an afternoon surge and a significant elevation in morning plasma prolactin levels until the fourth or fifth week compared to ovariectomized controls. Thereafter, a general decrease in plasma prolactin ensued until the level approached that of the ovariectomized controls.

All groups that received estradiol implants exhibited a significant increase in p.m. prolactin levels in the first week compared to a.m. levels, however, after the second week the a.m. and p.m. levels were not significantly different from each other. In the group with the No. 265 implants (figure, C, D) the levels in the morning increased abruptly until the second or third week and then leveled out for the remainder of the experiment. In contrast the animals with the No. 285 implants (figure E, F) demonstrated a continuous elevation in plasma prolactin in the morning throughout the duration of the experiment.

Discussion. The present experiments confirm our earlier observations that estrogen induces an afternoon prolactin surge[11] and potentiates the ether-induced elevation in plasma prolactin in the ovariectomized rat[12]. The present studies demonstrate, however, that these 2 effects of estrogen are initiated at different times. In ovariectomized rats the effect of ether inhalation on plasma prolactin levels in the morning and afternoon were not different; however, over the duration of the experiment there was a tendency for the morning levels to increase slightly. Whether this increase was induced by some change in the hypothalamic-pituitary axis following prolonged absence of estrogen is not certain.

In contrast to the ovariectomized rats, there was a difference in the morning and afternoon prolactin responses in the different estrogen-treated groups. In the polyestradiol phosphate-treated group an afternoon prolactin surge did occur within 1 week after estrogen administration and this afternoon response diminished over the duration of the experiment which is in agreement with the observations of Subramanian and Gala[13]. It appears that the magnitude of this afternoon surge was slightly diminished when the data are compared to levels in unanesthetized rats at the same time after estrogen treatment[13]. In this same group the response to ether in the morning was not obvious in the first week after estrogen treatment but by the second week the ether-response was greatly potentiated when compared to unanesthetized rats[13]. This potentiated response to ether confirms our earlier observations[12]. The animals with estradiol implants showed the same responses in the morning and afternoon in the first and second weeks as did the PEP-treated group. However, from the second week on the levels in the morning continued to increase dramatically whereas the afternoon levels increased slightly. This suggested that the afternoon surge levels were limited by some mechanism. Perhaps ether inhalation in the afternoon blunted the levels of prolactin as previously observed[17] or the high levels induced by ether in the morning limited the afternoon rise by the well documented short loop feedback mechanism[4, 18, 19].

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