recipient after transplantation. Figure 1 shows the plasminogen type of the donor, PLGN BBr (rare), and of the recipient, PLGN ABr, before renal transplantation. The Br (B rare) pattern consists of three major bands, two of which are shared with the B variant. The recipient did not lose the two major A bands in the 2 weeks after the transplantation. Figure 2 shows the plasminogen type of a donor, PLGN A, and of the recipient, PLGN B, before hepatic transplantation. In plasma samples obtained from the recipient after the liver transplant, the plasminogen type shows a change to that of the donor.

We conclude that most, if not all, plasminogen in human plasma is synthesized in the liver. These experiments confirm that virtually all plasma proteins except for the immunoglobulins are synthesized by the liver in vivo (*12*).

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Progesterone Administration in vivo Stimulates Release of Luteinizing Hormone–Releasing Hormone in vitro

Abstract. *The release of luteinizing hormone-releasing hormone (LHRH) from tissue from the mediobasal hypothalamic-anterior hypothalamic-preoptic area of prepuberal female rats was measured in a perifusion system. Measurements were also made of the concentrations of LHRH in these tissue fragments and of luteinizing hormone in serum obtained when the rats were killed. Four groups of immature rats were studied: intact, ovariectomized, ovariectomized and implanted with estradiol-containing capsules, and ovariectomized rats primed with estradiol and injected with progesterone. The release of LHRH from the tissue of ovariectomized animals was significantly less than that of intact females and was not modified when the ovariectomized rats received estradiol. However, there was a four- to fivefold increase in LHRH release from tissue of ovariectomized rats primed with estradiol when they were killed 6 hours after they received an injection of progesterone. The concentrations of LHRH in tissue and of luteinizing hormone in serum varied among groups and with the time of day that the animals were killed. The interactions among luteinizing hormone, gonadal steroids, and the photoperiod seem to set the appropriate conditions for neural processes triggering a complete and normal release of luteinizing hormone.*

Thirty years ago data were presented indicating a neural basis for the spontaneous release of luteinizing hormone (LH) in adult female rats (*1*). Subsequent evidence demonstrated that an estrogen-induced LH surge in female rats is synchronized with the photoperiod (*2*) and that this photoperiod-dependent LH surge occurs in immature female rats treated with steroids or gonadotropins (*3*). Furthermore, estrogen can induce LH release in intact but not in ovariectomized immature rats (*4*), whereas progesterone can facilitate the LH-releasing action of estrogen in both immature and adult rats (*5*). It has been assumed that the administration of estrogen or progesterone to immature rats trigger the release of LH-releasing hormone (LHRH) from the hypothalamus to the portal vessels and thus stimulate the release of LH from the anterior pituitary gland. This hypothesis is supported by the demonstration of increased concentrations of LHRH in the portal vessels of proestrous (*6*) and ovariectomized, steroid-treated rats (*7*). These observations prompted us to examine the release of LHRH from portions of tissue from the mediobasal hypothalamic-anterior hypothalamic-preoptic area (MBH-AHA-POA) (*8*) using a perifusion technique (*9*) and a specific radioimmunoassay for LHRH (*10*). Tissue samples were obtained from four groups of prepuberal rats killed either in the morning or afternoon of the photoperiod. We used four groups of rats: 1, intact; 2, ovariectomized; 3, ovariectomized and implanted with capsules containing 17β-estradiol; and 4, ovariectomized, implanted with 17β-estradiol, and injected with a single dose of progesterone (*11*). The effects of these hormonal manipulations in vivo on the release of LHRH from the MBH-AHA-POA tissue in vitro, on LHRH tissue concentrations, and on the concentrations of LH in serum were examined.

The release of LHRH from the tissue of ovariectomized animals was significantly less (*12*) than that of intact females and was not modified when ovariectomized rats received estradiol implants. Only the combination of estradiol implants and progesterone reinstated LHRH release to levels observed in intact females (see Fig. 1, a and b). None of the groups showed significant photoperiodic changes in LHRH release in vitro. High LHRH concentrations were measured in tissue from the rats in groups 1 and 4, with the highest LHRH values being obtained for the rats in these groups killed in the morning (see Table 1). The concentrations of LHRH from tissue of rats in groups 2 and 4 killed in the afternoon did not differ from the concentrations for rats in these groups killed in morning, but the LHRH concentrations for groups 1 and 3 were significantly reduced in rats killed in the afternoon (*13*). As expected, serum LH concentrations in the serum samples varied significantly as a function of time of death in all groups except group 2 (Table 1), with lower values being obtained from the rats killed in the morning.

These results show that hormonal manipulations in vivo in immature female rats influence (i) the concentrations of LHRH in tissue and (ii) the manner by which hypothalamic fragments perifused in vitro release this decapeptide. When compared to tissue from immature intact females, tissue from ovariectomized females showed a decrease in LHRH release in vitro, in spite of the fact that ovariectomy was followed by an in-

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Table 1. Concentrations of LHRH at the conclusion of superfusion for five experiments in which six MBH-AHA-POA units were used per experiment. The tissue from animals in the four groups were placed in superfusion chambers within 30 minutes of death in the morning. (1000 to 1100 hours) or evening (1630 to 1730 hours). Similarly, the LH in serum collected from these animals after they were decapitated was measured by radioimmunoassay (NIAMDD rat LH system). Numbers in parentheses indicate the numbers of samples assayed; results show means ± standard error.

Group	Tissue LHRH (pg/mg)		Serum LH (ng/ml)	
	a.m.	p.m.	a.m.	p.m.
1 (intact)	132.7 ± 17.5	42.5 ± 13.6*	6.3 ± 1.9 (N = 24)	39.1 ± 6.7* (N = 12)
2 (ovariectomized)	58.1 ± 5.0	72.0 ± 18.8	130.4 ± 21.6 (N = 13)	143.5 ± 21.9 (N = 17)
3 (ovariectomized plus estradiol)	87.5 ± 27.4	23.3 ± 9.9*	16.7 ± 3.1 (N = 18)	894.0 ± 199.7* (N = 18)
4 (ovariectomized plus estradiol and progesterone)	123.6 ± 11.1	94.1 ± 20.0	84.0 ± 23.9 (N = 22)	3553.8 ± 463.2* (N = 29)

*Significant ($P < .05$) a.m. to p.m. changes (Mann-Whitney U tests). We used one-way analyses of variance between hormone treatments within each time period. For LHRH concentrations, a.m.: groups 1 and 4 > group 2; group 1 > group 2. For LHRH concentrations, p.m.: group 4 > groups 1 and 3; group 2 > group 3. For serum LH, a.m.: groups 2 and 4 > groups 1 and 3. For serum LH, p.m.: groups 3 and 4 > groups 1 and 2; group 2 > group 1.

crease in plasma LH. The approximately twofold decrement in LHRH release was even more pronounced when these ovariectomized rats received an estradiol implant capable of producing a significant diurnal rhythm in blood LH (see Table 1). When immature rats primed with 17β-estradiol received an injection of progesterone, a four- to fivefold increase in LHRH concentration was measured in the perfusate. This high rate of LHRH release in vitro occurred in tissue from animals with either low plasma LH values (rats killed in the morning) or with extremely high values (rats killed in the afternoon).

There appears to be no simple relation between LHRH release in vitro and concentrations of LH in plasma. However, our results suggest some interesting possibilities. The ovariectomized immature rats in group 2 differed from those in the remaining groups by the absence of circadian response in all three dependent measures, namely, LHRH release, LHRH concentration, and serum LH. Relatively high plasma LH concentrations in these animals, compared to intacts, were not accompanied by either high LHRH concentrations in hypothalamic tissues or high LHRH release from these fragments. Therefore, these data suggest that the negative feedback action of estrogen on LH release is exerted at the pituitary level because ovariectomy was accompanied by increased release of serum LH in spite of decreased LHRH release in vitro. Ovariectomy has not produced consistently high levels of LHRH in portal vessel blood (*14*) and one report shows clearly that a decrease in LHRH occurs after ovariectomy (*7*).

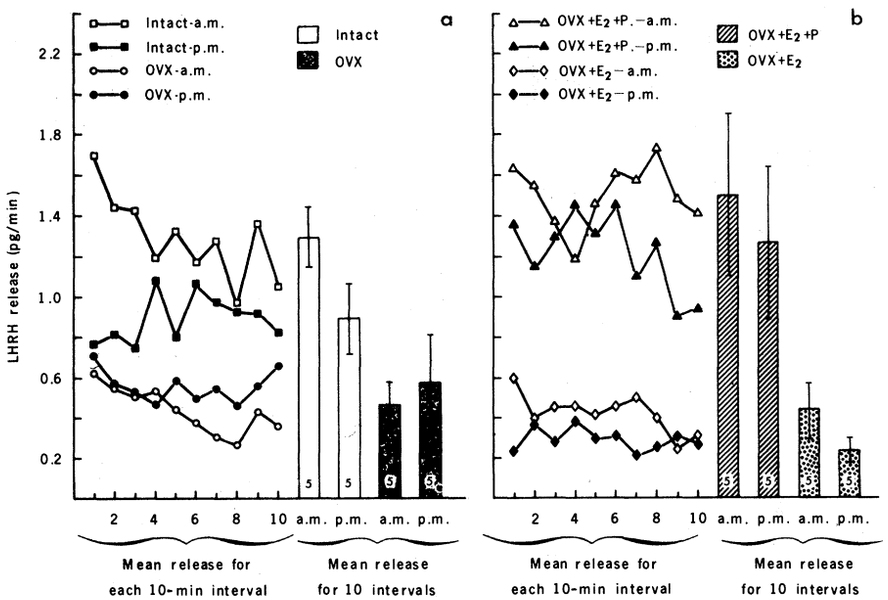

Fig. 1. The release of LHRH from MBH-AHA-POA units from 30-day-old female rats. The LHRH release was measured as picograms per minute per six MBH-AHA-POA units. The mean values for LHRH release are shown over values for each 10-minute interval and collapsed over the entire superfusion period (bar graph). (a) The rats were either ovariectomized (*OVX*) or subjected to sham surgery (*Intact*) at 28 days of age. Half the animals in each group were decapitated in the morning (0930 to 1030 hours) and the other half in the evening (1600 to 1700 hours). Each experiment was replicated five times. Bars indicate mean ± S.E. Intact-a.m. group > OVX group, $P < .05$. (b) The rats were ovariectomized and implanted with estradiol (E_2) Silastic capsules (*11*) (235 μg of 17β-estradiol per milliliter of oil) at 28 days of age. All animals received subcutaneously either 1 mg of progesterone ($OVX+E_2+P$) or a control injection of oil ($OVX+E_2$) 6 hours prior to decapitation either in the morning (0930 to 1030 hours) or evening (1600 to 1700 hours) with half the animals in each condition decapitated in each of the two time periods. Each experiment was replicated five times. Bars indicate mean ± S.E. OVX + E_2 + P group > OVX + E_2 group, $P < .05$. Animals were maintained on a light-dark cycle of 14:10 with lights off at 1900 hours.

It is possible that relatively high concentrations of blood LH decrease LHRH synthesis and release by way of a negative, short feedback loop controlling LHRH metabolism; this was suggested previously for immature (*15*) and adult rats (*16*) as well as rabbits (*17*). The animals in group 3 showed a clear-cut circadian release of LH which was not correlated with changes in LHRH release in vitro; curiously, compared to tissue from rats killed in the morning, there was a significant decrease in LHRH concentrations in the MBH-AHA-POA tissue from rats killed in the afternoon (Table 1). It is possible that the higher concentrations of LH in the afternoon provide a feedback to the hypothalamus and trigger a reduction in the synthesis of LHRH. It is also possible that low levels of tissue LHRH reflect greater LHRH release in vivo. When ovariectomized animals primed with estradiol were killed 6 hours after a progesterone injection a different picture emerged. Progesterone produced not only a four- to fivefold increase in LHRH release in vitro but also higher LHRH concentrations (fourfold) compared with those of tissue from rats in group 4 killed in the afternoon. It appears that progesterone was capable of partially overriding the negative feedback action of LH on LHRH metabo-

lism. Several investigators (*18*) have previously demonstrated that progesterone can stimulate LH release in ovariectomized animals primed with estradiol. This LH release is associated with increased LHRH in mediobasal hypothalamus and can be linked to activation of a noradrenergic system of neurons (*19*). Our results confirm their data and show that in a 6-hour period progesterone increases LHRH concentrations in MBH-AHA-POA tissue and stimulates higher release of the hormone relative to tissue from animals in group 3. These three factors, LH, gonadal steroids, and the photoperiod might interact to set the appropriate conditions for neural processes triggering a complete and normal surge of LH.

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11. Silastic capsules (Silastic medical grade tubing, 0.062 inch in inner diameter, 0.125 inch in outer diameter, and 10 mm in length) containing estradiol (235 μg/ml) in oil were incubated in physiological saline (37°C) for 24 hours before being implanted. Capsules were implanted immediately after ovariectomy in the dorsal neck region.
12. Data were analyzed by two separate two-way analyses of variance (time of day × hormone treatment). Fisher's least significance difference test was used for post hoc comparisons with $P < .05$ required for significance. Mann-Whitney U tests were used to evaluate the differences between a.m. and p.m. values for serum LH concentrations (Table 1).
13. In a separate control experiment, intact 30-day-old female rats were decapitated in the morning (0930 to 1030 hours), afternoon (1600 to 1700 hours), and night (2000 to 2100 hours) to determine LHRH concentrations in MBH-AHA-POA tissue before subjecting it to superfusion. The rats for these control experiments were kept under the same conditions as those used for the other experiments. The values (mean ± standard error, expressed in picograms per milligram; five animals per group) were 197.4 ± 36.8, 110.4 ± 30.2, and 131.5 ± 23.7, respectively. None of these values were significantly different, though LHRH concentrations tended to be lower in tissue from animals killed in the afternoon.
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Red Cochineal Dye (Carminic Acid): Its Role in Nature

Abstract. *Carminic acid, the well-known red dyestuff from cochineal insects (*Dactylopius *spp.), is a potent feeding deterrent to ants. This deterrency may be indicative of the natural function of the compound, which may have evolved in cochineals as a chemical weapon against predation. The behavior of an unusual predator is described—the carnivorous caterpillar of a pyralid moth* (Laetilia coccidivora)*—which is undeterred by carminic acid and feeds on cochineals. The animal has the remarkable habit of utilizing the ingested carminic acid for defensive purposes of its own.*

Before aniline dyes came into use in the latter half of the 19th century, one of the most important red dyes in the textile industry was carminic acid (Fig. 1C), an anthraquinone extracted from scale insects (Coccidae) of the genus *Dactylopius*, the so-called cochineals (*1*). Although in commercial use, no biological function had been demonstrated for this substance (*1-3*). Other quinones, such as the benzoquinones and naphthoquinones discharged from the defensive glands of certain insects, millipeds, and opilionids (Fig. 1, A and B), are potent feeding deterrents to predators, including predaceous insects such as ants (*4*). Assuming that an anthraquinone might be similarly defensive, we undertook bioassays which showed that carminic acid is indeed potently deterrent to ants. The results of these tests are presented, together with an account of the behavior of a predaceous caterpillar that is undeterred by carminic acid and feeds on cochineals, and is able to put the ingested anthraquinone to defensive use of its own.

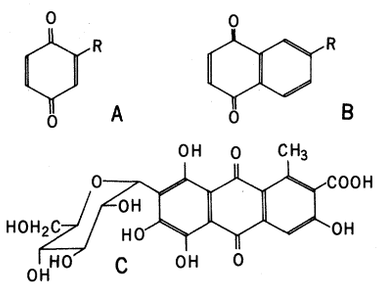

Fig. 1. (A) 1,4-Benzoquinones [for example, R = H, CH_3, C_2H_5 in defensive secretions of certain beetles (*25*)]. (B) 1,4-Naphthoquinones [for example, R = H, CH_3 in defensive secretion of an arachnid (*26*)]. (C) Carminic acid, the anthraquinone of cochineal insects.

Our observations were made on *Dactylopius confusus*, a cochineal species commonly found on prickly pear cacti (*Opuntia* spp.) in Florida. Aggregations of *Dactylopius* are conspicuously white (Fig. 2A), as a result of the fluffy investiture of waxy powder and silken threads that characteristically cloaks their bodies (*5*). This "wool" is thought to be defensive (*1*, *6*). Newborn cochineals and males (the only winged form) are devoid or nearly devoid of wool (*7*) and are distinctly red because of the carminic acid in their bodies (Fig. 2B). Cloaked individuals have similar red bodies, as is evident when they are plucked bare with forceps. Carminic acid is present in the blood and muscles of the immature insects and adults, as well as in the eggs and embryos within gravid females (*1*). The carminic acid content of reproduc-

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