were inappropriately elevated throughout the GnRH replacement period for undetermined reasons.

In a subsequent series of experiments, performed for another purpose, which differed from the foregoing only in that the post-GnRH infusion ovariectomies were not performed, nine of ten animals ovulated and exhibited normal plasma progesterone patterns during the luteal phase.

Most of the experimentally induced ovulatory menstrual cycles occurred in the presence of elevated plasma prolactin concentrations resulting from the hypothalamic lesions (*5*). These ranged as high as 500 ng/ml, compared with a normal mean of 20 ng/ml. There was no significant difference between the plasma prolactin concentrations of the animals that responded to GnRH with ovulatory menstrual cycles and those that did not, which indicates that a hyperprolactinemia of this magnitude, in the rhesus monkey, does not interfere with ovarian responses to physiologic gonadotropin stimulation nor with the actions of GnRH.

Our findings are consonant with the hypothesis that the ovary is the principal timer of the primate menstrual cycle and that estradiol controls gonadotropin secretion by acting directly on the pituitary gland, the intermittent release of GnRH by the hypothalamus being a permissive, but necessary, component of this control system. This conclusion has recently been reinforced by preliminary studies conducted in collaboration with Ferin and his colleagues, wherein experiments identical to the ones described here but performed on rhesus monkeys with transected pituitary stalks (*8*) rather than hypothalamic lesions yielded essentially identical results.

There thus seems to be a major difference in the hypothalamic control of the monkey menstrual cycle on the one hand and of the rat estrous cycle on the other. In the latter, a bolus of GnRH, discharged into the pituitary portal vasculature in response to the action of estrogen on the central nervous system, is clearly necessary for the induction of the preovulatory gonadotropin surge (*9*). In the rhesus monkey, however, such an increment in hypothalamic activity is not required to initiate this phenomenon.

E. KNOBIL
T. M. PLANT, L. WILDT*
P. E. BELCHETZ†, G. MARSHALL
Department of Physiology,
University of Pittsburgh,
School of Medicine,
Pittsburgh, Pennsylvania 15261

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* Present address: Universitäts-Frauenklinik, 53 Bonn-Venusberg, West Germany.
† Present address: Endocrine Unit, St. Bartholomew's Hospital, West Smithfield, London EC1, England.

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Experimental Induction of Puberty in the Infantile Female Rhesus Monkey

Abstract. *Normal ovulatory menstrual cycles were initiated in prepubertal female rhesus monkeys by the infusion of gonadotropin-releasing hormone for 6 minutes once every hour. When this regimen was discontinued, the animals promptly reverted to an immature state. These findings permit the conclusion that neither adenohypophysial nor ovarian competence is limiting in the initiation of puberty and suggest that this process depends on the maturation of the neuroendocrine control system that directs the pulsatile secretion of gonadotropin-releasing hormone from the hypothalamus.*

In all primate species studied, including the human, attainment of reproductive competence is a late maturational event. Although all other aspects of adenohypophysial secretory activity proceed at the adult level long before the initiation of puberty, the hypothalamico-hypophysial system that governs gonadotropin secretion in the adult is nonfunctional during the prepubertal period (*1*, *2*). In this context, the sexually immature female rhesus monkey bears a striking resemblance to adult females with lesions in the arcuate region of the mediobasal hypothalamus (*3*). These lesions, which leave other aspects of adenohypophysial function essentially intact, abolish gonadotropin secretion, presumably by interrupting the normal pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Normal ovulatory 28-day menstrual cycles can be reestablished in such animals by an unvarying physiological GnRH replacement regimen consisting of hourly intravenous infusions of the decapeptide administered by suitably programmed infusion pumps (*4*). We have investigated the question of whether the same hypothalamic hormone replacement regimen that subserves normal ovarian function in adults with hypothalamic lesions can also initiate and maintain normal ovulatory menstrual cycles in immature females.

We used six prepubertal female monkeys that were born in our colony. The monkeys were 11 to 15 months old at the beginning of the study; this is approximately 14 months before menarche and about 20 months before ovulatory menstrual cycles are first observed (*1*, *5*). Each monkey was fitted with a cardiac catheter connected to an infusion withdrawal device that enabled us to administer GnRH and obtain blood samples without having to restrain the animals (*6*). This device, originally used in adult monkeys weighing 5 to 8 kg, was adapted to the infantile animals (1.6 to 2.3 kg) and was well tolerated. It did not interfere with their normal activity, weight gain, or behavior. The GnRH was delivered by Harvard pumps programmed to infuse 1 μg of GnRH per minute for 6 minutes once every hour (*7*). This unvarying infusion regimen was maintained for 93 to 253 days.

Blood samples (1.0 to 2.5 ml, depending on the frequency of sampling) were taken midway between two infusions of GnRH, usually between 0900 and 1200 hours every third day during the first 17 days of GnRH administration and daily

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thereafter. The concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, and progesterone in plasma were determined by radioimmunoassays (*8*). At the end of the GnRH infusion period the catheters were removed and blood samples were taken by femoral puncture. Thirty-one days after the termination of the GnRH infusion Silastic capsules filled with crystalline estradiol (*9*) were implanted subcutaneously and left in place for 7 days, achieving plasma estradiol concentrations between 400 and 1100 pg/ml, to assess the positive feedback action of the steroid.

The GnRH infusion regimen, which subserved normal ovarian cycles in adults with hypothalamic lesions, initiated ovulatory menstrual cycles in all six infantile animals studied. The resulting time courses of circulating gonadotropins and ovarian steroids were indistinguishable from those observed during spontaneous menstrual cycles in adult monkeys. Two representative experiments are illustrated in Fig. 1. Within a few days after the initiation of the GnRH replacement regimen, gonadotropins became detectable in the peripheral circulation. In response to this increment in LH and FSH concentrations, plasma estradiol increased from previously undetectable levels to those characteristic of the follicular phase of the adult menstrual cycle. As a consequence of final follicular maturation, estradiol reached peak concentrations in excess of 200 pg/ml, within the range occurring before ovulation in adult monkeys (*10*). These initial estradiol peaks, however, did not induce ovulatory gonadotropin surges in any of the six animals studied. A second estradiol peak occurred approximately 14 days later; this increase did induce surges of LH and FSH in three of the six monkeys. Two animals responded to a third increase in estrogen and one to a fourth with unambiguous discharges of gonadotropin. These gonadotropin surges induced ovulation and corpus luteum formation as indicated by the appearance of progesterone in the plasma. The resulting time course of circulating progesterone was characteristic of the luteal phase of adult menstrual cycles, and terminated in menstruation. In three of four prepubertal females in which more than one ovarian cycle was observed the intervals between succeeding gonadotropin surges were 27 to 31 days, which is within the normal range of menstrual cycle length in adult animals of our colony. In the remaining monkey this interval was 102 days. After the discontinuation of the GnRH infusion regimen the animals reverted to the prepubertal state as evidenced by the decline of gonadotropins and ovarian steroids to undetectable levels and the failure of estradiol administration to induce surges in gonadotropin.

Menarche eventually occurred in five of these animals 4 to 10 months after the cessation of GnRH infusion, when they were between 24 and 27 months old. One animals is still premenarchial at the age of 26 months, 11 months after the discontinuation of the GnRH infusion.

These results demonstrate that neither the pituitary nor the ovary is limiting in

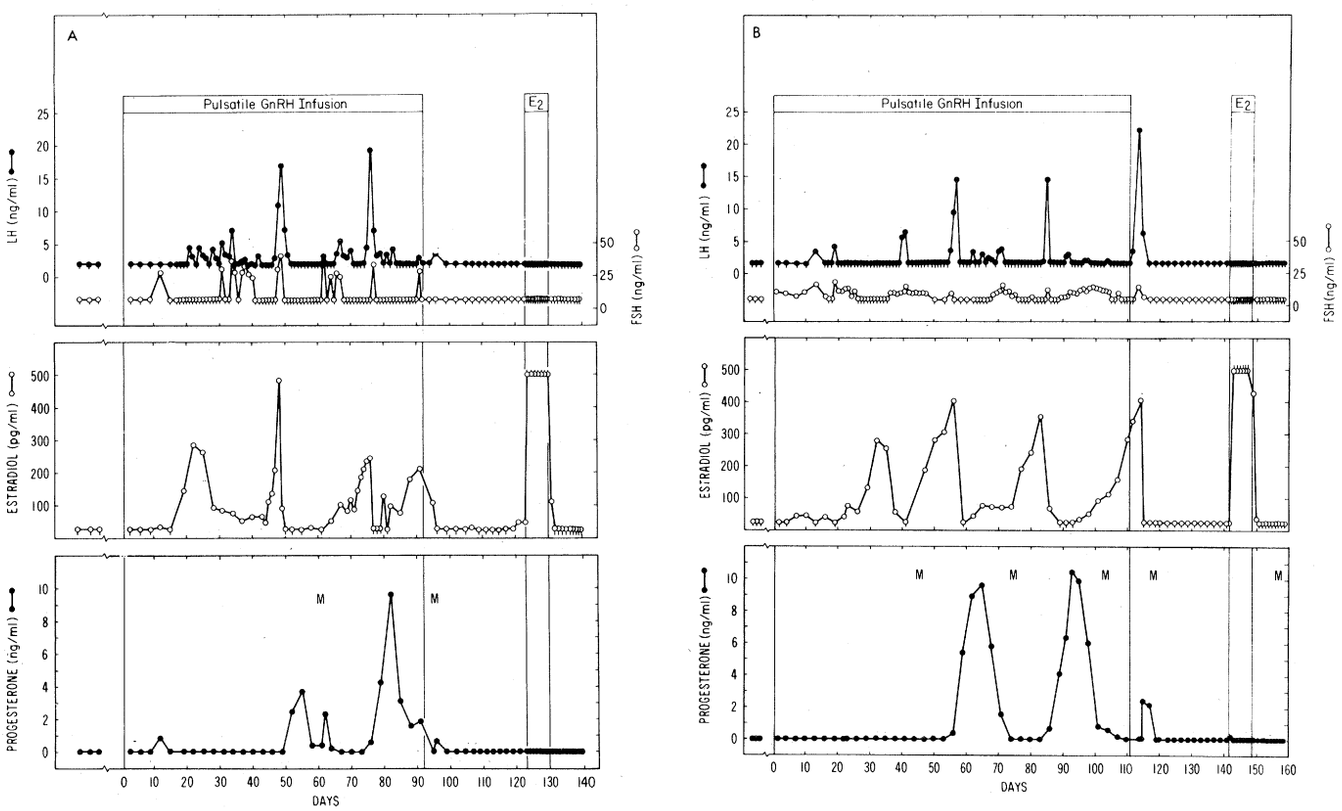

Fig. 1. Induction of ovulatory menstrual cycles in two immature monkeys by the administration of a GnRH replacement regimen (1 μg/min for 6 minutes once every hour). The GnRH infusion was initiated on day 0 and was continued as shown by the open horizontal bars. Gonadotropins and ovarian steroids were undetectable in control samples taken before the start of the GnRH infusion. The first increase in estradiol was ineffective in eliciting gonadotropin surges. The interval between the gonadotropin surges induced by subsequent increments of estradiol was 27 days in one animal (A) and 28 days in the other (B). In one animal (B) a gonadotropin surge occurred in response to the estradiol increment 2 days after the termination of the GnRH infusion (*11*). Progesterone secretion from the corpus luteum induced by this gonadotropin surge was only transient, however, and could not be sustained in the absence of hypophysiotropic stimulation. The horizontal open bars labeled E_2 indicate the implantation of estradiol-containing Silastic capsules. The small vertical lines underneath some data points denote hormone concentrations below the sensitivity of the respective radioimmunoassays. Estradiol concentrations surpassing 500 pg/ml are depicted with vertical lines above the respective data points. The occurrence of menstruation is indicated by *M*.

the initiation of puberty in the rhesus monkey and that a period of induced normal pituitary and ovarian function in sexually immature animals does not necessarily entrain continued ovarian cyclicity. Furthermore, our findings suggest that puberty is normally initiated by the activation of hypothalamic mechanisms that control the pulsatile release of GnRH into the pituitary portal circulation. What causes this activation after a long period of prepubertal quiescence, however, remains to be elucidated.

L. WILDT*
G. MARSHALL
E. KNOBIL

Department of Physiology,
University of Pittsburgh,
School of Medicine,
Pittsburgh, Pennsylvania 15261

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Present address: Universitäts-Frauenklinik, 53 Bonn-Venusberg, West Germany.

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Histolysis Initiated by "Lymph Gland" Cells of *Drosophila*

Abstract. *In late third-instar larvae and young pupae of* Drosophila hydei, *the "lymph gland" releases cells that migrate through the hemocoel and selectively attach to the basal laminae of transient larval organs. The lymph gland cells contain many inclusions—in particular, dense bodies, which appear to be secreted at the attachment sites. Since this process occurs before histolytic changes become apparent, it is suggested that the lymph gland cells initiate histolysis.*

In holometabolous insects, two contrasting processes occur concomitantly during metamorphosis: histolysis of the transient larval organs and histogenesis of the prospective imaginal organs. Although much is known about the development and histogenesis of imaginal disks, little attention has been directed toward the process of histolysis. It is still poorly understood.

Two different views have been advanced to explain histolysis of larval organs during metamorphosis. First, Pérez (*1*) described the breakdown of larval tissues in *Calliphora* as the result of a direct attack by phagocytic hemocytes. Second, in *Lucilia* (*2*), *Drosophila* (*3*), *Sarcophaga* (*4*), and *Calliphora* (*5, 6*), initial autolytic changes of the larval organs, including the fragmentation of muscles ("sarcolyte" formation), were described as occurring independently of hemocytes and before the latter invade the tissue.

In a recent review, Rizki (*7*) emphasized that the larval "lymph gland" of *Drosophila* is an organ of uncertain function. Srdić and Gloor (*8*) studied this organ in *Drosophila hydei* and concluded that its role is at the beginning of metamorphosis. In this report we show that the lymph gland plays an essential role in pupal histolysis.

Morphology and behavior of larval lymph gland cells were studied in *D. hydei* by light and electron microscopy, in vivo and after removal and transplantation of lymph glands labeled in vitro with [^{3}H]uridine (1 hour; 10 μCi per microliter of Ringer solution), followed by autoradiography of 1-μm Epon sections of host flies at different times after transplantation. The tissue was fixed and prepared for electron microscopy by standard techniques. To identify lysosomes, the test for acid phosphatase was carried out (*9*).

In *Drosophila* embryos (*10*) and newly hatched larvae (*8*), the lymph gland consists of a few cells on either side of the aorta behind the brain. Less than 1 day after hatching, three pairs of cell groups form. These grow slowly during larval life in a manner comparable to the growth of imaginal disks (*8*). (In *D. hydei* larvae we observed a 100-fold linear increase in the size of the lymph gland between the first and the late third instars.) Twenty hours before pupariation, the lymph gland begins to release its cells, called oenocytoids (*11, 12*). At the end of the larval stage, 10 percent of all cells floating in the hemolymph are oenocytoids (*8*). The release process continues until 2 days after pupariation, when all cells are liberated and the lymph gland disappears.

Inside the lymph gland, the cells form a relatively compact mass surrounded by a distinct basal lamina (Fig. 1A). All cell nuclei are euchromatic and contain a large nucleolus. The cytoplasm is characterized by a well-developed, rough endoplasmic reticulum, abundant Golgi elements, and two types of vesicles. Besides many electron-transparent vesicles, a strikingly large number of dense bodies are present. These show a strongly positive reaction in the acid phosphatase test, which indicates that they are lysosomes (Fig. 1C). The dense bodies are often arranged in rows and form extensive cisternae, which branch at their periphery. These structures are similar to the GERL (Golgi endoplasmic reticulum lysosomes) in granule-forming vertebrate secretory cells (*13*). In lymph glands of middle third-instar larvae, the same cells contain few, if any, dense bodies. However, small vesicles with dense cores are often present at the forming face of the Golgi stacks (Fig. 1B). Such vesicles are also observed in lymph gland cells at more advanced stages and in oenocytoids in the hemocoel. They seem to represent an early stage of dense-body formation.

Lymph glands from late third-instar larvae were labeled as before and injected into the posterior parts of larvae of the same age. Autoradiography of 1-μm Epon sections from the hosts enabled us to identify, in the hemolymph, single cells from the donors. About 10 hours after transplantation, labeled cells were present throughout the body cavity of each host. The labeled cells selectively attached to the basal lamina of transient larval tissues; for example, the abdominal and thoracic muscles, tracheae, and fat body (Fig. 1, D and E). In the hosts, the basal lamina of the muscles often ap-

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