(*11*), the incubation was continued for 30 minutes to ensure that all susceptible group B antigens were converted to H activity. We then tested cells from the primary reaction mixture and obtained the same results. The enzyme solution was removed after centrifugation and the packed cells were washed four times with 45 ml of phosphate-buffered saline (PBS) to remove any remaining traces of the enzyme preparation. The cells were left in the last wash for 15 minutes to ensure equilibration to *p*H 7.3. After centrifugation, the cells were resuspended in 12.5 ml of PBS to a final volume of 15.5 ml. Three 5-ml portions, each containing about 1 ml of converted cells, were taken up in preweighed syringes, and the weight of cells to be administered was measured. Transfusion was performed quantitatively by several back-and-forth rinsings of each syringe with the recipient's blood. A portion of the remaining 0.5 ml of cells was used as a standard to determine the total amount of radioactivity administered.

During the ensuing 49 days blood samples were taken and their radioactivity was measured to determine the survival of the transfused cells. Using data on the first blood samples, which were taken 15 minutes after transfusion, we calculated the blood volumes of the three participants (Table 1) and found them to be within the normal range (*12*). The results, which reflect the amount of radioactivity initially administered as compared with the amount found in the circulation, are very similar for all three recipients. This indicates that there was no selective destruction of enzymatically converted cells by the immune systems of the O and A individuals in the first few minutes after transfusion. The amount of radioactivity was essentially unchanged in samples of whole blood taken at 15, 30, and 60 minutes (Table 1), and the plasma in these samples was devoid of ^{51}Cr, demonstrating that rapid destruction of converted cells was not occurring at these time points. These values were averaged and taken to represent 100 percent survival.

Over 95 percent of the converted cells were present in the circulation after 24 hours (Table 1). At least 50 percent of the converted cells remained in the circulation for 30 to 33 days. This is in accord with published values for normal survival (*13*). Finally, the three survival curves (Fig. 1) have essentially the same shape. They did not show a marked downward shift in slope to indicate an increase in the rate of cell destruction due to an immune response (*14*).

These results are the first indication that small quantities of enzymatically converted group B erythrocytes can survive normally in recipients whose immune systems would not tolerate untreated cells.

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5 October 1981

Luteal Phase Defects Induced by an Agonist of Luteinizing Hormone–Releasing Factor: A Model for Fertility Control

Abstract. *Subcutaneous injection of 50 micrograms of a luteinizing hormone–releasing factor agonist (LRF agonist) for three successive days at the time of menstruation in normal cycling women induces a shortened luteal phase with suboptimal concentrations of circulating estradiol and progesterone. This luteal phase defect follows a reduced concentration of follicle-stimulating hormone during the follicular phase and a resulting inadequate follicular maturation. Since a short luteal phase is associated with an endometrium not conducive to implantation, administration of the LRF agonist at the onset of menstrual cycle may prove to be a practical and novel approach to fertility control.*

The "spontaneous" occurrence of short or inadequate luteal phases of the menstrual cycle in humans (*1*) and monkeys (*2*) has received increasing recognition as a significant cause of infertility and repeated early abortions (*3*). The endometrium in women with such luteal phase defect is known to develop inadequately with respect to both its morphology and function as a result of impaired secretion of estradiol (E_2) and progesterone. A normal predecidual reaction usually does not occur and normal nidation is thereby impeded (*4*).

In monkeys there is evidence that such luteal defects are the consequence of a deficiency in follicle-stimulating hormone (FSH) which results in suboptimal folliculogenesis. However, there is no direct evidence in women that FSH deprivation during the early follicular phase of the menstrual cycle is causally related to the sequence of impaired follicular maturation followed by luteal dysfunction.

Since infertility is a dominant feature of luteal phase dysfunction (*1–4*), pharmacologically induced luteal defects would appear to offer considerable potential as a method of contraception. Prolonged infusion of luteinizing hormone–releasing factor (LRF) or daily administration of long-acting LRF agonists is known to induce pituitary desensitization resulting in lowered concentrations of circulating gonadotropin (*5*). We therefore explored the possibility that the administration of an LRF agonist very early in the follicular phase might cause inadequate folliculogenesis and subsequent luteal dysfunction.

Five normal women (ages 22 to 39 years) with menstrual cycles of 28 days volunteered for this study. In each subject, a control cycle and a treatment cycle were studied by daily measure-

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ment of luteinizing hormone (LH), FSH, E_2, and progesterone (*6*). Treatment cycles included the subcutaneous administration of 50 μg of [D-Trp^6,Pro^{9}NEt]-LRF (*7*) daily for the first 3 days after the onset of menstrual flow. This long-acting LRF agonist is approximately 140 times as potent as the native decapeptide LRF in women (*8*).

The LRF agonist, as administered in this study, first induced a transient rise in the circulating concentrations of LH and FSH as well as the gonadotropin-dependent ovarian secretion of E_2. This was followed by depressed levels of FSH, lower than those in the control cycles ($P < .01$, analysis of variance) during the remainder of the follicular phase (Fig. 1). In contrast, LRF agonist administration did not produce demonstrable changes in serum LH concentrations. After an initial transient increase, serum E_2 was not significantly influenced until the last 4 days of the follicular phase when E_2 concentrations in the treated cycles were found to be significantly lower ($P < .01$, analysis of variance) than in the control cycles. These hormonal changes induced by LRF agonist were associated with a 9-day delay in the onset of the midcycle gonadotropin surge, thus creating a prolonged follicular phase (Table 1). At the surge, the peak levels attained for LH, FSH, and E_2 were significantly lower ($P < .01$, $< .005$, and $< .05$, respectively) in the treated cycles than in the control cycles (Fig. 1).

Table 1. Mean differences (± standard error) between cycle length and duration of follicular and luteal phases in control cycles and cycles treated with LRF agonist. Administration of the LRF agonist resulted in a shortened luteal phase in four subjects and an inadequate luteal phase in one subject.

Cycle	Follicular phase (days)	Luteal phase (days)	Cycle length (days)
Treatment	23.2 ± 1.2	9.2 ± 0.2*	33.4 ± 0.7
Control	14.2 ± 0.2	14.0 ± 0.3	28.2 ± 0.2

*One subject (Fig. 2) showed an inadequate luteal phase (*1*), as defined by abnormally low E_2 and progesterone concentrations with a luteal phase of normal length. The result for this subject is excluded in the analysis of the mean duration of shortened luteal phases.

These follicular phase aberrations appear to be extended into the luteal phase. Although LH and FSH concentrations during the luteal phase were similar to those in the controls, a marked reduction in serum E_2 and progesterone concentrations was observed in the treated cycles. Of particular importance is the observed early failure of luteal function as evidenced by the fall in E_2 and progesterone on the eighth and ninth days following the gonadotropic surge, about 5 days prior to luteal regression in the controls. Consequently, the mean luteal phase was shortened from 14.0 to 9.2 days (Table 1 and Fig. 1). In one subject (Fig. 2) with a luteal phase of normal length in the treated cycle, E_2 and progesterone concentrations were unequivocally lower than those in the control cycle (inadequate luteal phase), indicating an impairment of steroidogenesis and functional inadequacy of the corpus luteum.

The antecedent follicular phase abnormalities, common to the treated cycle in all subjects, may be causally related to the ensuing luteal dysfunction. The mechanism that accounts for this extended effect on the entire ovarian cycle after early exposure to an LRF agonist is not clear. Unlike the effect of the LRF agonist in the rat, the LRF agonist in the human probably does not act directly on the ovary, as evidenced by the lack of effect of LRF agonist on steroidogenesis in isolated human granulosa cells and the absence of binding of LRF or its agonist to the luteal cells in monkeys (*9*). The reduced FSH secretion, and thus the inappropriate ratio of FSH to LH, may result from an initial excessive stimulation and subsequent "desensitization" of the gonadotropes by the long-acting LRF agonist (*5*). This preferential reduction of FSH secretion may also be explained by the initial increase in E_2, the inhibiting action of which is greater on FSH than on LH (*8*). In any event, this relative FSH deprivation may lead to a relative deficiency of aromatase, an

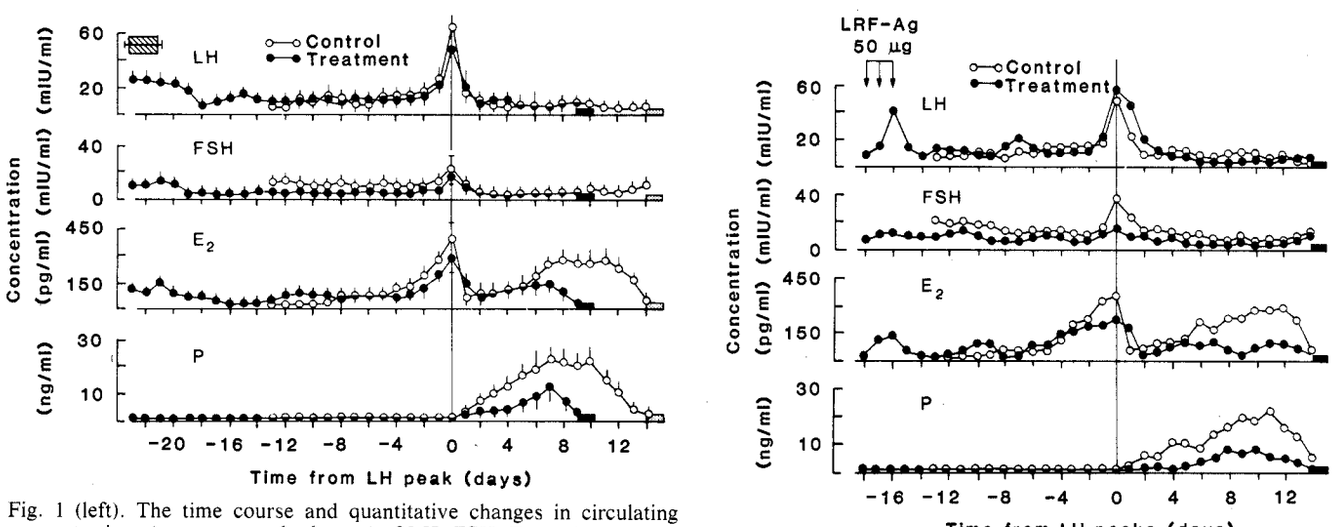

Fig. 1 (left). The time course and quantitative changes in circulating concentrations (mean ± standard error) of LH, FSH, E_2, and progesterone (*P*) during the menstrual cycle of four normal women treated with LRF agonist (hatched box, top left) on the 3 days after onset of menstruation, and compared to the preceding control cycles. Data were centered around the midcycle gonadotropin peak (day zero). Onset of menstrual flow occurred on day 9 in the treated cycles (solid boxes) and day 14 after the midcycle surge in the control cycles (dotted boxes). Fig. 2 (right). Pattern of circulating concentrations of LH, FSH, E_2, and progesterone (*P*) during the control and LRF agonist–treated cycles in one subject (Table 1). This subject showed a prolonged follicular phase (19 as opposed to 14 days) with reduced FSH concentrations, reduced concentrations of FSH and E_2 during the midcycle surge, and a normal luteal phase length but reduced E_2 and progesterone concentrations during the entire luteal phase of the treated cycle. Data were centered around the midcycle gonadotropin surge (day zero). (Solid boxes indicate the period of menstruation.)

FSH-dependent enzyme that converts androgen to estrogen in the granulosa cell (*10*). The selective binding of FSH to granulosa cells not only generates FSH receptors and aromatase activity but also induces LH receptors (*11*). Thus, FSH deficiency during the follicular phase may result in lowered numbers of LH receptors for the luteal phase, and thereby diminish the luteotropic activity of LH on luteal cells. This interpretation is consistent with the observation that in women with a short luteal phase, treatment with human chorionic gonadotropin (hCG) frequently fails to rescue luteal function (*12*). In addition, a decreased hCG sensitivity of luteal cells in vitro has been observed in monkeys with follicular phase FSH deficiency induced by porcine follicular fluid (*12*).

Although LRF agonists are also effective luteolytic agents, the presence of a narrow window of maximum sensitivity during the luteal phase of the cycle detracts from the practical application of those agonists as contraceptives (*13*). Luteolysis does not occur with the mode of administration used in our studies; fertility is probably curtailed because of the early regression of the corpus luteum which occurs 1 to 2 days before the expected date of nidation (days 8 to 9 after the LH surge) (*14*), and the resulting inadequate development of the estrogen-progestin–dependent endometrium. The convenience of timing administration of the LRF agonist at the onset of menstruation represents a major advantage for practical application to fertility control and offers the possibility for providing a "once a month pill."

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31 August 1981; revised 16 October 1981

Actin Distribution Patterns in the Mouse Neural Tube During Neurulation

Abstract. *With the use of antibodies to actin and indirect immunofluorescent techniques regions of increased actin concentration were demonstrated first in basal and later in apical areas of mouse neuroepithelial cells. These patterns of staining corresponded to shape changes observed in cranial neural folds as they initially elevated from the neural plate and later moved toward the midline.*

Microfilaments have long been thought to play a role in the process of neurulation. Ever since Baker and Schroeder (*1*) described the presence of apical microfilaments in amphibian embryos, these structures have been implicated as the driving force responsible for apical constriction of neuroepithelial cells (*1–4*). This apical constriction in turn leads to the creation of "flask-shaped" cells that aid in elevation of the neural folds resulting in the process of neurulation (*2*).

Evidence for the contractility of these microfilaments is derived from their morphological similarities to other such filaments known to be composed of nonmuscle actin (*5, 6*). Additional evidence that neuroepithelial microfilaments contain actin has been provided by demonstrating the typical arrowhead pattern when such filaments from chick neuroepithelial cells are reacted with heavy meromyosin (*7*). Although these data strongly suggest that neuroepithelial microfilaments contain actin and play a contractile role, further characterization of actin distribution patterns in neurulating tissues would serve to substantiate this hypothesis. We have studied the distribution of actin in cranial folds of mouse embryos at different stages of neurulation using antibodies to actin and indirect immunofluorescent methods. Mouse embryos were selected because cranial neural folds are larger and assume a more complex shape in mammals than in anurans or urodeles. Thus, actin distribution patterns may also be more complex in mammals.

Antibodies to actin were prepared by injecting rabbits with highly purified chicken gizzard actin (*8*). These antibodies are specific for actin according to the following criteria: (i) the gizzard actin was purified by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (SDS-PAGE); (ii) the antibody bound to homogeneous gizzard, skeletal muscle, and cardiac actin when these were tested in a solid-phase assay; (iii) the antibody showed the expected immunofluorescent patterns such as stress fibers in fibroblasts and typical I-band staining in muscle fibers; and (iv) the antibody bound only to actin on overlays of nitro-

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