The Timing of Birth

A hormone unexpectedly found in the human placenta turns out to influence the timing of delivery. This and related findings could yield much needed ways to prevent premature labor

by Roger Smith

Over the past 30 years, doctors have become increasingly skilled at saving premature babies—those born before the 38th week of gestation instead of at the more typical 40 weeks. Unfortunately, premature infants who survive are often afflicted by breathing difficulties, cerebral palsy, intellectual handicaps and other problems.

Six to 8 percent of all newborns arrive before term. Of those, perhaps half are delivered early because of spontaneous premature labor. In theory, then, interventions that prevented such labor could spare a great many infants from death or lifelong disability.

Yet prevention has failed entirely. The reason? Until recently, scientists have had little understanding of the biological mechanism that controls birth timing and thus of how to keep that mechanism from operating inappropriately.

In the past few years, researchers in several centers, including my laboratory at the University of Newcastle in Australia, have gained a much clearer sense of the controls on birth timing. With this information in hand, we are beginning to explore exciting new ideas for avoiding premature labor and for delaying delivery until the fetus is mature enough to thrive outside the womb.

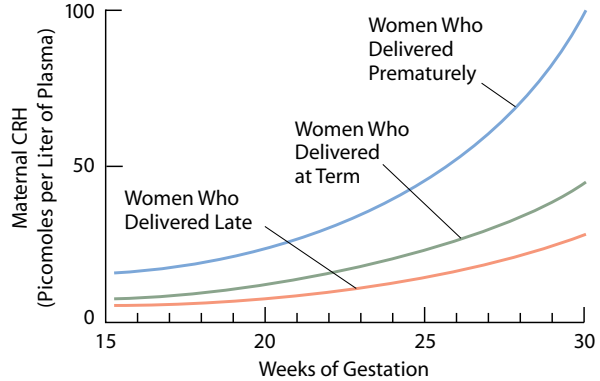
The newly deciphered mechanism actually determines more than the exact moment of birth. It regulates parturition: the uterine, cervical and other changes that make labor possible. Parturition, which usually takes place in the last two weeks of human pregnancy, culminates in delivery.

Springboard to Progress

The recent progress in deciphering how parturition is controlled has built on many insights into parturition itself. Specifically, scientists have known for some time that throughout most of gestation the uterus is essentially a relaxed bag of disconnected smooth muscle cells. This bag is sealed at the bottom by a tightly closed ring—the cervix—which is kept firm and inflexible by tough collagen fibers. These structural features are maintained by progesterone, a steroid hormone that the placenta secretes into the mother's circulation from early in pregnancy. Yet the placenta also secretes estrogen, a steroid that opposes progesterone and promotes contractility.

At first, maternal estrogen levels are relatively low, but over time they rise. Parturition typically begins when the balance of power shifts so that the estrogen and other forces favoring contraction override those blocking it.

Notably, as maternal estrogen levels soar, cells of the uterine muscle (the myometrium) synthesize a protein called connexin. Connexin molecules then move to the cell membrane and form junctions that electrically link one muscle cell to another. Wired into a network, the muscle cells become able to undergo coordinated contractions. At the same time, estrogen prods the myometrial cells to display large numbers of receptors for oxytocin, a hormone (made in the brain) that can increase the force of uterine contractions and induce labor in a receptive uterus.



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TIMING OF DELIVERY in humans appears to be determined largely by the rate at which the placenta releases a protein called corticotropin-releasing hormone (CRH) into the maternal and fetal circulations. This "placental clock" was uncovered by measuring CRH levels in the blood of nearly 500 women as their pregnancies progressed. In general, those with the highest levels early on (by 16 to 20 weeks) had the fastest clocks and were most likely to deliver prematurely.

As the uterine muscle prepares for labor, estrogen also promotes the manufacture of chemicals called prostaglandins by placental membranes overlying the cervix. The prostaglandins induce production in the cervix of enzymes that digest its collagen fibers; the enzymes thereby convert the cervix into a malleable structure that will dilate progressively, and finally open, as the infant's head presses against it during labor.

While all these changes are occurring, yet another hormone—cortisol, made by the fetal adrenal gland—ensures that the infant's lungs undergo the final changes required for breathing air. In particular, at high levels cortisol leads to production of substances that remove water from the lungs and enable them to inflate.

Even as investigators accumulated knowledge of estrogen's role in parturition, they continued to be baffled by the nature of the switch (in the fetus or in the mother) that activates placental estrogen secretion. For practical and ethical reasons, biochemical changes occurring in the developing human fetus, in the placenta and in the pregnant woman are extremely difficult to study closely. Therefore, biologists sought, and found, many clues to the regulation of parturition in experiments performed on other large mammals, especially sheep.

Sheep System Emerges

By the mid-1980s, such studies—initially pioneered in the 1960s by Graham C. ("Mont") Liggins of the National Women's Hospital in Auckland, New Zealand—had discerned the basic regulatory mechanism in sheep. The same mechanism operates in most mammals.

At some point near the middle of gestation in sheep, the hypothalamus of the developing fetal brain begins to secrete a hormone called corticotropin-releasing hormone (CRH), which induces the pituitary gland, at the base of the brain, to secrete adrenocorticotropin (ACTH) into the fetal circulation. ACTH instructs the fetal adrenal gland to make cortisol. This hormone, in turn, activates enzymes in the placenta that convert progesterone to estrogen. Consequently, secretion of progesterone into the mother's circulation falls, and that of estrogen rises. When cortisol levels in the fetus become quite high, they also facilitate maturation of the lungs.

In the nonpregnant ewe, as in the nonpregnant human, cortisol is part of what is known as a negative-feedback system. The cortisol feeds back to the hypothalamus and pituitary to dampen the release of ACTH and to reduce cortisol manufacture, so that cortisol levels remain stable instead of rising endlessly. Toward the end of gestation, however, cortisol in the fetus lacks this braking effect (for reasons that are still unexplained). As a re-

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sult, fetal levels of ACTH and cortisol, and hence maternal estrogen levels, rise throughout the last part of the sheep pregnancy. Ultimately, the mother's estrogen concentrations become high enough, and the progesterone levels low enough, for parturition to commence.

Disappointingly, as this tidy scheme was being pieced together, work in humans revealed that a central feature did not operate in people. As was the case in sheep, fetal cortisol apparently did help the lungs to mature in humans; cortisol-like drugs given to a woman in premature labor did reduce the likelihood that the baby would suffer breathing difficulties. Yet cortisol had no effect on parturition and did not induce pregnant women to go into labor.

Today the collected evidence suggests that CRH drives fetal cortisol production and placental estrogen manufacture, and thus parturition, in humans as well as in sheep. Strikingly, though, most of this CRH in humans comes not from the fetal brain but from the placenta. In addition, CRH induces placental estrogen secretion through a markedly different pathway than is the case in sheep and in most other nonprimate mammals.

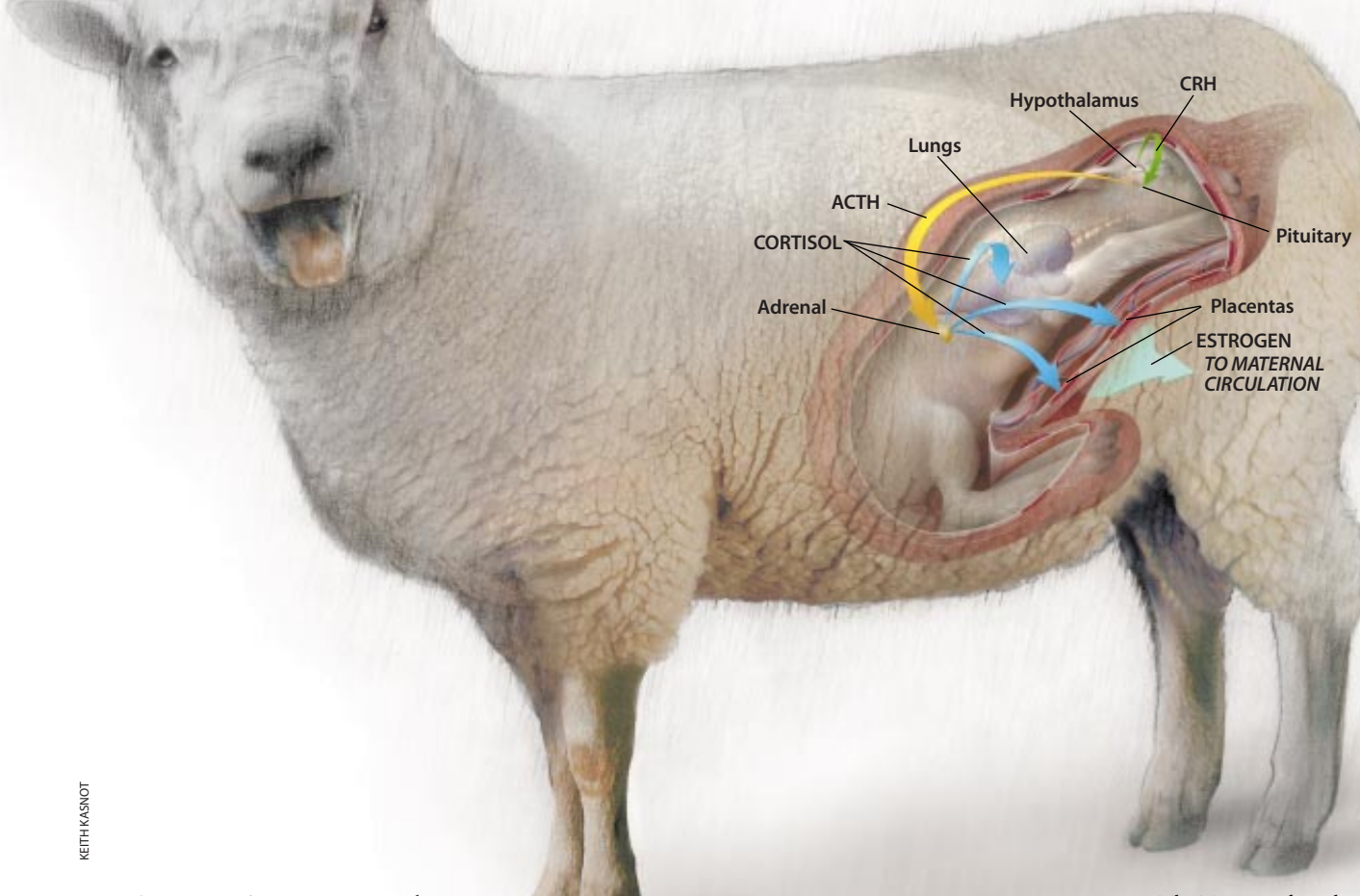
A Human Placental "Clock"

Hints that placental CRH was important in human parturition first appeared in the 1980s. Early in that decade Tamotsu Shibasaki of Tokyo Women's Medical College and his colleagues made the surprising discovery that the human placenta contained CRH. This revelation was astonishing because the brain was thought to be the sole producer.

In the 1980s as well, various teams demonstrated that CRH from the placenta became detectable and rose sharply in the mother's blood toward the end of pregnancy and then disappeared—signs that it might serve some role in parturition. Equally suggestive, in the second half of the decade, clinicians in England and the U.S. found that women who went into premature labor had higher blood levels of CRH at delivery than did women who were tested at the same week of pregnancy but who did not deliver before term.

At about the same time, a young medical school graduate named Mark McLean joined my group as a Ph.D. student. As his thesis, he undertook a more rigorous test of the possible link between CRH and the onset of parturition. He had blood samples drawn from almost 500 women all through their pregnancies, measured CRH and then looked to see whether the levels correlated with the timing of delivery. The project was time-consuming and took many years, but finally, in the mid-1990s, the day arrived when the analyses were complete.

EVENTS LEADING TO LABOR are controlled by the fetal brain in sheep and in most other mammals. Near the middle of gestation, the hypothalamus of the brain begins to secrete CRH, which causes the pituitary to release adrenocorticotropin (ACTH). ACTH, in turn, induces the fetal adrenal gland to secrete cortisol. Cortisol causes the sheep's multiple placentas to convert progesterone to estrogen, which enters the mother's blood. At high levels, estrogen initiates parturition: preparation of the uterus and cervix for labor and delivery. Meanwhile cortisol also promotes fetal lung maturation.



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At first glance, the results did not seem surprising. They confirmed that maternal blood concentrations of CRH increase as gestation advances, and they added the discovery that the levels rise exponentially throughout pregnancy. But as we gazed at the results, something much more intriguing became apparent: CRH values at 16 to 20 weeks of pregnancy (the earliest our tools could detect them) roughly predicted when the women would give birth. What is more, mothers with the highest levels were most likely to deliver prematurely, and those with the lowest levels were most likely to deliver past their official due dates.

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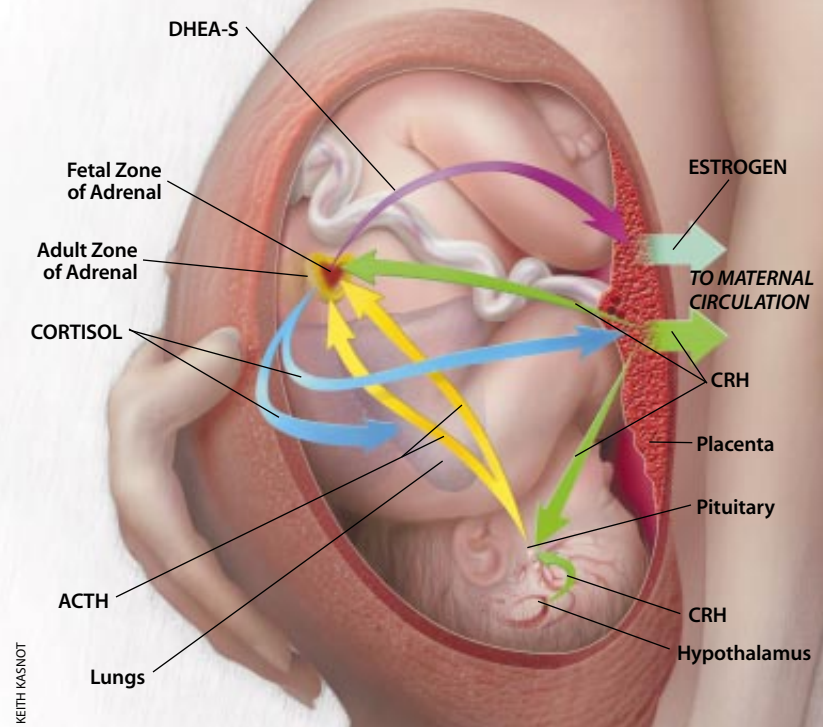
In other words, McLean had uncovered the presence of a "clock" that was set early in pregnancy and that controlled the speed with which a pregnancy advanced. The clock could be read (albeit rather crudely) by looking at the amount of CRH in a mother's blood. It now seems likely that the rate of CRH production is itself controlling the duration of pregnancy, although at the time we had to consider that placental CRH manufacture might be a mere by-product, or marker, of some other process that was truly orchestrating parturition.

The results were more exciting than we could have anticipated. Beyond adding basic insight into human parturition, they raised the possibility that by assessing CRH levels relatively early in



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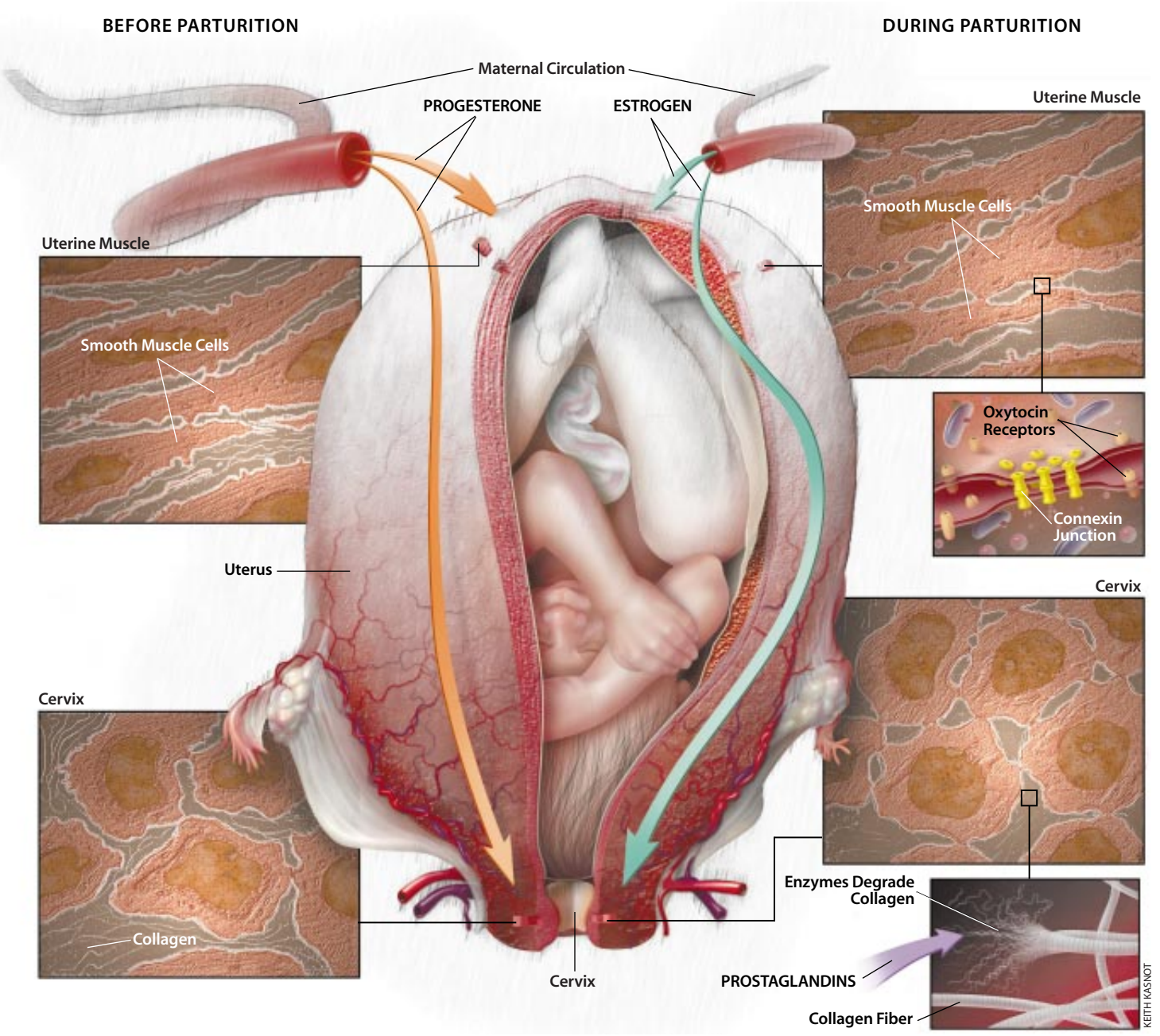
CONTROLS ON PARTURITION in humans differ from those in sheep. Notably, much CRH comes from the placenta, not solely from the fetal brain. CRH acting on the fetal pituitary leads to cortisol manufacture by the fetal adrenal gland, just as occurs in sheep, but this cortisol does not induce the placenta to make the estrogen required for parturition. Instead it mainly promotes maturation of the fetal lungs and helps to maintain CRH manufacture by the placenta. Estrogen is made after CRH from the placenta and ACTH from the fetal pituitary stimulate the fetal adrenal gland to secrete dehydroepiandrosterone sulfate (DHEA-S), which the placenta converts to estrogen.

pregnancy, physicians might be able to identify women at risk for premature spontaneous labor. Such identification would warn these expectant mothers that they need to be monitored closely and to give birth at facilities with neonatal intensive care units. Moreover, the ability to find women at risk would enable scientists to conduct systematic trials of new preventive therapies, comparing treatment against nontreatment in equivalent populations of women known to be in danger of going into early labor.

CRH analyses are not yet done routinely, in part because the best methods for measurement and the most useful time to perform the tests are still under evaluation. Such assays may well be used in the future, though. I should note that CRH levels vary considerably from woman to woman and that normal or low levels do not guarantee protection against early labor. In some cases, infections of the baby or other events can result in premature delivery even when CRH levels are not initially elevated.

Why do many researchers now think that placental CRH plays a crucial role in regulating human birth timing and is not merely a marker of some more



ESTROGEN HAS MANY EFFECTS on the uterus and cervix of pregnant mammals. For most of pregnancy, maternal progesterone ensures that uterine muscle cells are relaxed and that tough collagen fibers keep the cervix firm (*details at left*). Eventually, though, sharply elevated estrogen levels cause uterine muscle cells to display receptors for oxytocin, a hormone that "tells" the cells to contract during labor (*top details at right*). Estrogen also prods the cells to make connexins, which electrically wire the cells together, enabling them to contract in synchrony during labor. Meanwhile chemicals called prostaglandins lead to production in the cervix of enzymes able to digest collagen (*bottom details at right*); these enzymes make the cervix malleable.

powerful regulatory process? They have been swayed by studies that over the past 10 years have revealed a molecular cascade by which that hormone could well lead to the estrogen increase required for parturition.

How CRH Regulates Parturition

When it became clear that parturition was regulated by a somewhat different process in people than in sheep, many teams began to study closer relatives of humans, namely, nonhuman primates. Experiments on monkeys and apes are more complicated to perform than studies on sheep, but those animals are the only ones whose placenta, like that of humans, produces CRH during pregnancy.

In the late 1980s my group and, independently, that of Robin S. Goland of Columbia University turned to baboons. Each of us found that in contrast to the ever rising levels of CRH in human mothers-to-be, levels in baboons go up rapidly early in pregnancy and then drop back to moderately elevated levels, which remain constant for the rest of gestation. This result led us exactly nowhere until one day in 1996, when I was sitting in a lecture hall at the International Congress of Endocrinology, watching a presentation on the development of the fetal adrenal gland by two leading experts on baboon pregnancy: Eugene D. Albrecht of the University of Maryland and Gerald J. Pepe of Eastern Virginia Medical School.

By then, reproductive scientists already knew that the adrenal gland of the primate fetus is different from that of the sheep fetus and of the sheep and primate adult. Instead of being divided into a central medulla and an outer cortex that can secrete cortisol, the primate fetal adrenal has no medulla and a two-part cortex, most of which consists of an internal area called the fetal adrenal zone. The smaller, outer part of the cortex still produces cortisol, but the fetal adrenal zone makes a steroid hormone with a tongue-twister name: dehydroepiandrosterone sulfate, or DHEA-S for short.

Also in contrast to the findings in sheep, the primate placenta lacks the cortisol-responsive enzymes needed to make estrogen from progesterone. Instead the placenta constructs the estrogen needed for parturition out of DHEA-S. (This activity explains why progesterone levels do not fall at the end of human pregnancies as they do in sheep; the human placenta cannot cannibalize progesterone to make estrogen, so the progesterone continues to survive and make its way into the maternal circulation.)

Albrecht and Pepe's data indicated that the relative size of the baboon's fetal adrenal zone—the size compared with that of the fetus—grew in a very interesting way. Whereas the relative size in humans and rhesus monkeys peaked near the end of gestation, that of the baboon was largest in midgestation. Later the zone grew more slowly, and it disappeared after delivery.

As I watched their slides, I noticed that the pattern paralleled the rise and fall of placental CRH in the pregnant baboon. Most likely, CRH from the placenta was directly or indirectly controlling the secretion of DHEA-S from the baboon's fetal adrenal zone. Could it be doing the same thing in humans and thereby causing the late rise in estrogen secretion by the placenta?

I could not wait to begin testing that idea. On my return to Newcastle, my colleagues and I quickly showed that human fetal adrenal tissue contains receptors for CRH, an indication that it is responsive to signals from that hormone. Then, with Robert B. Jaffe and Sam Mesiano of the University of California at San Francisco, we established that human fetal adrenal zone cells do indeed respond to CRH by making DHEA-S, not cortisol. (They also make DHEA-S in response to ACTH from the pituitary.)

Other Roles for CRH

For parturition and labor to occur only when the fetus is ready for life outside the womb, the master controller of parturition would have to ensure not only that estrogen levels were high before delivery but also that enough cortisol was made for lung maturation. Placental CRH apparently meets that requirement, too. As Joseph A. Majzoub of Harvard Medical School has proposed, placental CRH in the fetal circulation could very well stimulate the release of ACTH from the fetal pituitary and thereby stimulate the adrenal gland to produce the cortisol needed for lung maturation. In other words, placental CRH is well situated to coordinate fetal development with parturition and thus to assure that the baby is ready for delivery when labor begins.

Other work indicates that CRH, in addition to prompting estrogen production by the placenta and cortisol manufacture by the fetal adrenal gland, acts directly on the uterus and cervix. In so doing, it may augment the changes induced by estrogen or may sometimes compensate for inadequate production of estrogen.

For example, a British team has some evidence that maternally circulating CRH, like estrogen, enhances the concentration of prostaglandins in the cervix and thus facilitates its softening. And researchers in England and Italy have demonstrated in strips of human uterine muscle that incubation with CRH can potentiate the contractions induced by other substances, including the hormone oxytocin.

The way seems open for the creation of tests able to identify pregnant women at high risk of premature labor.

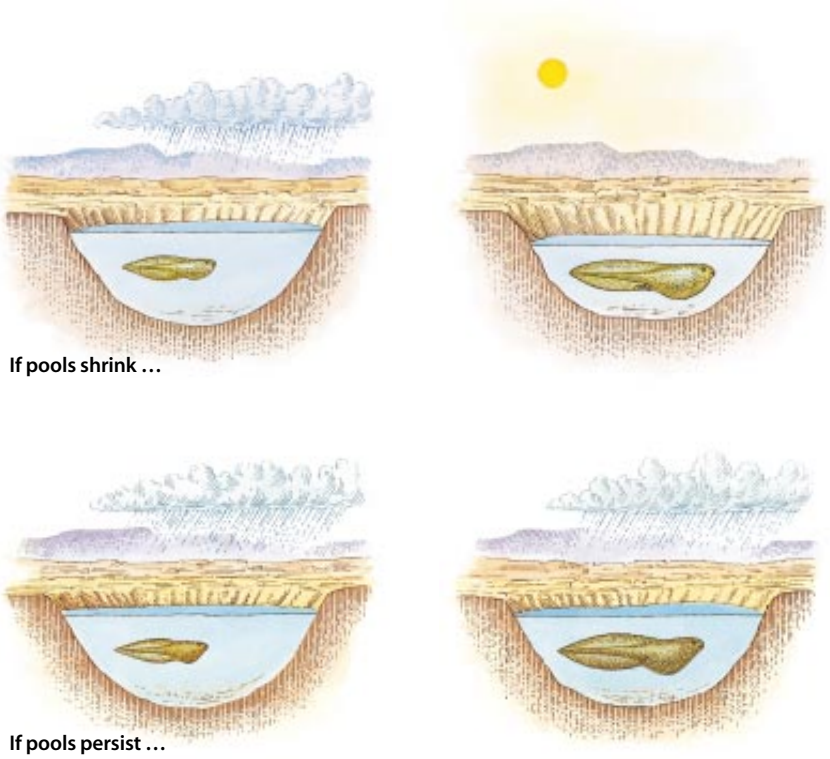
Further, Edward W. Hillhouse and Dimitri Grammatopoulos of the University of Warwick in England report that several different forms of the CRH receptor can appear on uterine muscle cells and that the mix of receptors changes during parturition. Early in pregnancy, receptors that are bound by CRH react by causing intracellular reactions that normally promote the relaxation of muscle cells. Later the receptors on the laboring uterus promote contraction.

What, though, causes the placenta to make CRH, and what controls how much is made? These fascinating questions remain unanswered. Majzoub and Bruce G. Robinson of the University of Sydney Medical School in Australia have, however, demonstrated that once the placenta begins to release CRH, cortisol can support its continued secretion. Among the factors that could conceivably cause one person to manufacture more CRH than another from the start are differences in the mother's nutrition early in pregnancy and subtle variations in the genetic makeup of the CRH-producing cells in the placenta.

An Evolutionary Clue from Toads

When did CRH first become a key regulator of birth timing? No one has an answer yet, but a recent discovery in amphibians hints that CRH has had that role for a good chunk of evolutionary time. The Western spadefoot toad, a desert dweller, lays its eggs in pools formed by rain. If the pools shrink from lack of precipitation, the tadpoles from those eggs quickly metamorphose into small toads (*top row*). If the pools persist, the tadpoles develop more slowly and grow large before metamorphosing (*bottom row*).

Robert J. Denver of the University of Michigan has found that the environmental effects are mediated by CRH, which is produced at a higher rate in the rain-deprived group. This finding suggests that reliance on CRH to control development might have evolved well before mammals appeared. —*R.S.*



It appears that in humans, placental production of CRH, which is made from about the 12th week of gestation, begins slowly. At first it stimulates the growing fetal adrenal zone to secrete small amounts of DHEA-S, which the placenta converts to estrogen. Meanwhile CRH from the placenta, and probably from the fetal brain, signals another part of the adrenal gland to secrete some cortisol into the fetal circulation. This cortisol, as Majzoub and Robinson have suggested, further stimulates placental release of CRH, thus forming a "feed-forward" system in which CRH production never shuts down. Instead the circuit operates relentlessly. When critical thresholds of CRH, estrogen, prostaglandins and probably other factors are all passed, the uterus and cervix undergo many changes, and labor begins.

More Complexity

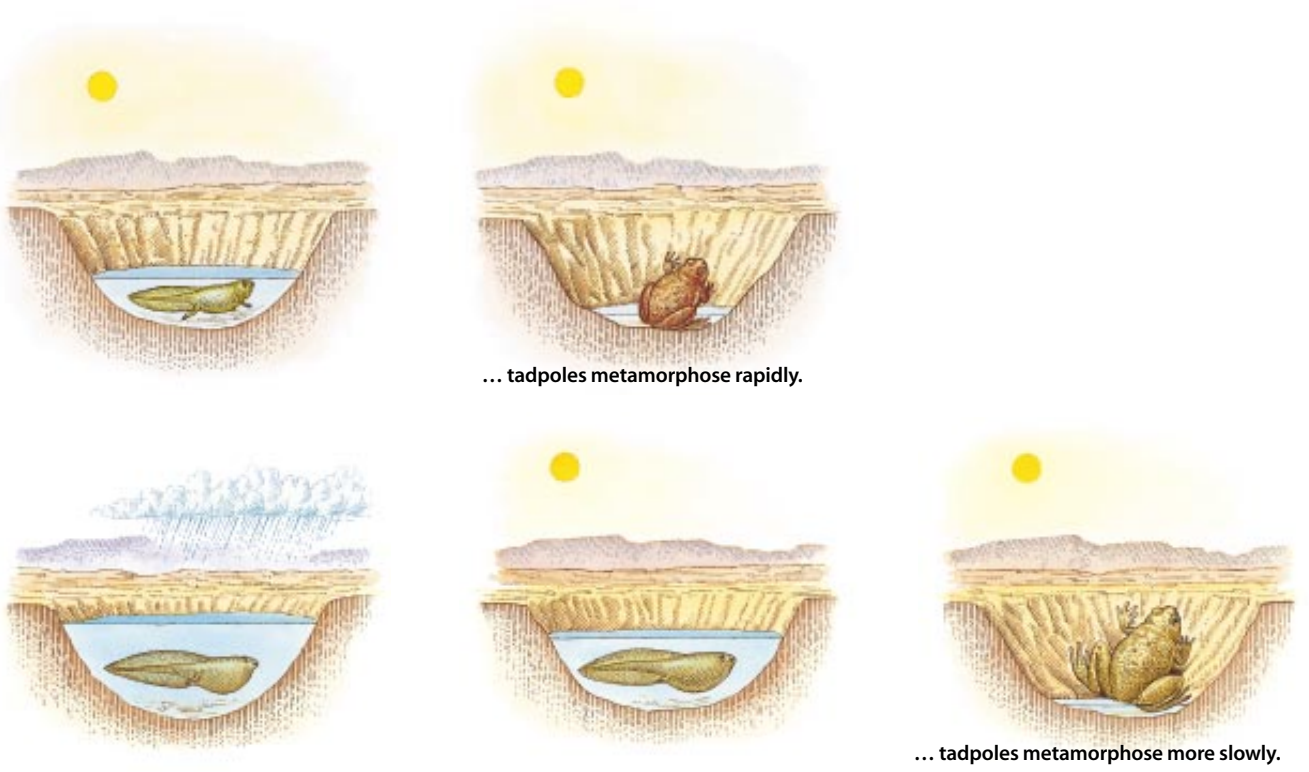
Still, this scenario is incomplete. Factors other than the self-perpetuated, feed-forward circuit can influence parturition and delivery. The size of the fetus may have an effect. A mature baby will stretch the uterine muscle, and stretching can intensify the muscle's responsiveness to contractile stimulation.

The nutritional state of the fetus may also play a part, according to I. Caroline McMillen of the University of Adelaide in Australia. She has suggested that in sheep, nutrient deprivation can precipitate delivery. Such deprivation may occur when a fetus grows large and the placenta ages. Supporting evidence for this concept has also been noted in humans. Pregnant Jewish women observing the fast of Yom Kippur, and thus reducing the nutrient supply to their fetuses, show a peak in delivery rates that is not observed on Yom Kippur in nonfasting Bedouin women living in the same region. Perhaps the stress of inadequate nutrition activates the fetal stress system, which involves production of CRH by the hypothalamus in the fetal brain. CRH release by the hypothalamus would be expected to boost ACTH and cortisol levels and thus to amplify the activity of the entire parturition-inducing circuit.

The finding that estrogen and CRH both can magnify contractility of uterine muscle suggests even more complexity. We have presented one sequence of events that seems to regulate parturition, but aspects of the control mechanism might be redundant. The direct action of CRH on the uterine muscle might, for instance, play a minor part most of the time but take on a more critical role if estrogen manufacture is impaired. Such redundancy may serve as more than a safety net. As Stuart A. Kauffman of the Santa Fe Institute has pointed out, redundancy in complex systems allows such systems to evolve. If change in one redundant pathway improves the operation of the system, the change will be retained; if the alteration is detrimental, an ostensibly extraneous pathway could prevent the change from becoming deadly.

To address how the system regulating human parturition evolved, my colleagues and I at Newcastle are collaborating with E. Jean Wickings and others at the International Center for Medical Research of Franceville in Gabon. In these experiments, we are trying to determine roughly when primates acquired more intricate controls on parturition than those that operate in other mammals. We are also attempting to gain a handle on why that change occurred.

One thing is clear, however. Reliance on CRH as a major director of development has a long evolutionary history, possibly dating to a time before mammals joined amphibians and other animals on the earth. Robert J. Denver of the University of Michigan has evidence, for example, that CRH influences the speed at which tadpoles of the desert-dwelling Western spadefoot toad (*Scaphiopus hammondii*) develop and



ROBERTO OSTI; SOURCE: LEIF SAUL

metamorphose into toads [*see box beginning on opposite page*].

As fascinating as the evolutionary questions are, the overriding reason for investigating parturition is to find ways to prevent preterm labor. Improved understanding of the regulatory system in humans has suggested a range of therapeutic options.

Prospects for Intervention

As a case in point, we and others are exploring the value of CRH inhibitors as preventives of premature labor. In collaboration with George P. Chrousos's team at the National Institutes of Health, my colleagues and I at Newcastle have recently shown that a CRH antagonist called antalarmin can delay delivery in sheep. If such antagonists prove safe and effective in nonhuman primates, trials in people will surely follow. Human trials of oxytocin antagonists are under way, and preliminary data in women imply that prostaglandin blockers might be helpful as well.

Work on identifying women at risk is also proceeding. Aside from exploring the value of measuring CRH levels in maternal blood, scientists are seeking other markers of trouble. In my laboratory we are assessing whether untoward rises in collagen-degrading enzymes in the cervix can identify expectant mothers who are about to enter labor too early.

In an interesting sidelight, my team has shown that the level of maternal CRH may be a useful indicator of whether artificial induction of labor will be successful. Expectant mothers with high levels of CRH are more likely to respond to induction procedures than are those whose levels are low.

The way now seems open for the creation of tests able to identify pregnant women at high risk of premature labor and for the development of agents able to modify the production of CRH or to otherwise slow the placental clock that controls the timing of delivery. Such application-oriented efforts will be informed by many results from more basic research. In concert, both kinds of endeavors hold promise for achieving a precious goal: giving more babies the chance to realize their full potential, free of the physical and educational handicaps too often associated with preterm birth. SA

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Further Reading

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