Dunbar, an anti-zona vaccine developer at Baylor College of Medicine in Houston. Several researchers are now trying to identify small peptides that exert a contraceptive effect, but do not provoke an autoimmune reaction.

Many researchers, however, argue that a better way of avoiding autoimmune pathology would be to produce a vaccine for women based on antigens from sperm, which will be foreign to females. And there's good reason to think it should be possible to produce a safe and effective anti-sperm vaccine: A small proportion of infertility in both women and men seems to be due to the production of anti-sperm antibodies.

The strategy poses a formidable technical hurdle, however. There are millions of sperm in a single ejaculate, and to hit this large and moving target, researchers must ensure that antibodies are secreted into the female reproductive tract by its mucosal lining. Despite recent progress by vaccinologists in stimulating this "mucosal immunity" (*Science*, 9 September, p. 1522), researchers are still some ways from being able to manipulate such responses at will.

But there are signs that this hurdle will not be insurmountable. In a 1988 paper in *Nature*, a group led by the husband-and-wife team of Paul Primakoff and Diana Myles of the University of Connecticut showed a 100% contraceptive effect in female guinea pigs immunized with a sperm protein called PH-20. The effect was reversible, and there was no need to couple PH-20 to an immunogenic molecule, as the protein is readily recognized as foreign by the female immune system. Since then, the Connecticut researchers have concentrated on cloning the PH-20 genes from a range of species including monkeys and humans and working out how to make large quantities of species-specific PH-20 using recombinant DNA technology. Now, they're ready for the next big test: repeating their guinea pig experiments in primates.

Getting a better response

To date, only one candidate anti-sperm molecule has been shown to depress fertility in primates. This is a form of the enzyme lactate dehydrogenase (LDH) that seems to be unique to sperm. It was discovered three decades ago by biochemist Erwin Goldberg of Northwestern University in Evanston, Illinois. In an experiment due to be published next year in *Biology of Reproduction*, Goldberg found that immunization with the sperm-specific LDH caused a 78% reduction in fertility in a group of female baboons, compared with unvaccinated controls. When the animals were "rested" for a year, says Goldberg, their fertility returned to normal.

That's an encouraging result, but the efficacy of Goldberg's vaccine remains far below that of existing contraceptives. "For a vaccine to be viable," says Primakoff, "you have to get it above 95% efficacy." With the notable exception of Primakoff's guinea pig experiments, however, contraceptive vaccines have struggled to meet that requirement. Indeed, even Talwar's vaccine does no good for the 20% of women who fail to produce large amounts of anti-hCG antibodies following immunization.

Contraceptive vaccine developers, however, are confident that response rates will be improved. The problem is thought to lie with the activation of immune system cells called "helper" T cells, which provoke the immune system's antibody factories, B cells, into action. In an immune response, foreign antigens first become bound to glycoproteins encoded by genes in the major histocompatibility complex (MHC), which are carried by so-called antigen presenting cells. Helper cells can then recognize these antigens and stimulate antibody production. However, each MHC glycoprotein binds to a different range of antigens. So given natural genetic variation in the MHC, women who respond poorly to Talwar's vaccine are probably simply producing a range of MHC glycoproteins that fail to bind effectively to it.

"[Contraceptive vaccines] won't be very attractive if [people] have to come in frequently to get shots."
—Vernon Stevens

If so, Talwar says it should be possible to boost the response rate by replacing the toxoid carrier used to stimulate helper T cells with a cocktail of "promiscuous T cell epitopes"—peptides that bind to a wide range of different MHC glycoproteins. "The eventual aim is to have close to 100% of women responding," he says.

Nevertheless, refining even Talwar's vaccine into a usable product will take time, and he warns against expecting the widespread introduction of an anti-hCG vaccine before the end of the decade. But as WHO's Griffin points out: "The need for new methods is going to be as great—if not greater—in 10 years as it is now."

–Peter Aldhous

Peter Aldhous is a writer at New Scientist.

PERINATAL RESEARCH

Stopping Premature Births Before It's Too Late

Too much, too soon, is one way to describe the current situation with premature births. "A national disgrace," is how Robert Garfield, director of Reproductive Sciences at the University of Texas Medical Branch in Galveston, refers to it. Figures compiled by the Centers for Disease Control show that in the United States more than 10% of babies are born prematurely. According to a 1985 National Academy of Sciences report, some $5 billion is spent on premature infants, mostly for high-tech neonatal care. However, the money is no guarantee of survival: A 1985 study published in the *British Medical Journal* estimates that premature births account for 85% of all early infant deaths. And the babies who live are prone to a host of problems, ranging from blindness and cerebral palsy to poor school performance. "The problem is just devastating, especially to parents," says Garfield. But now there are some signs that help is on the horizon.

Traditional efforts to prevent premature births have focused—with limited effectiveness—on stopping labor contractions that have already begun. So researchers are developing a new paradigm, trying to pinpoint the causes of premature labor in hope of nipping the problem in the bud. "The real issue," says perinatal researcher and infectious-disease specialist James McGregor of the University of Colorado Health Sciences Center in Denver, "is to prevent premature birth and avoid the whole issue entirely."

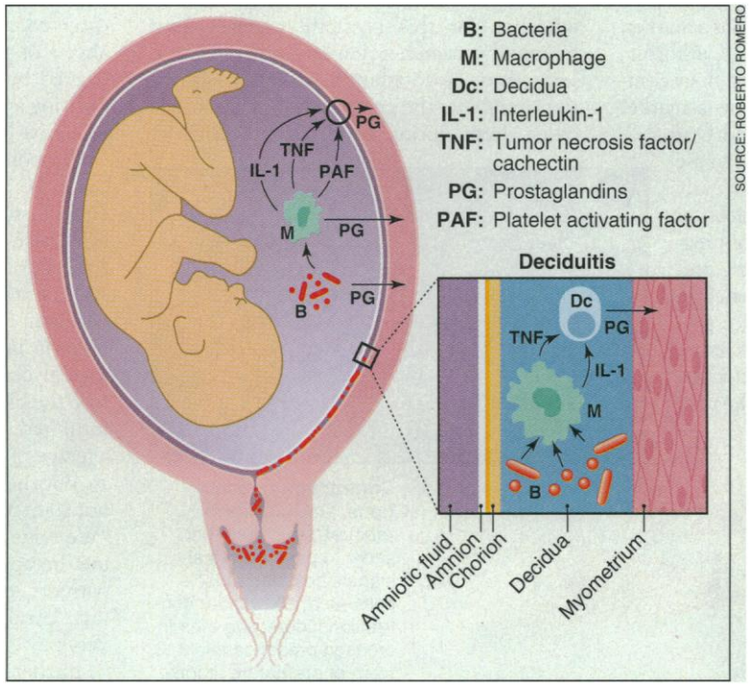
Much of this work focuses on identifying and treating uterine infections, which some researchers consider to be a major cause of premature births. In other efforts, scientists are looking for biochemical markers that signal that labor is about to begin. A clinical trial of the efficacy of using one such marker has already started. And finally, scientists are taking a new look at the physiology of uterine contractions, hoping to find ways to prevent them if they begin prematurely. "I'm very excited about the possibilities," says parturition researcher Charles Lockwood of the Mount Sinai School of Medicine in Manhattan. "We're attacking the problem with a vengeance from several standpoints."

Such a wholesale effort may be necessary, because the causes of premature birth are many and varied, ranging from inadequate blood flow to developmental disorders to allergies.

And, although there is some disagreement about it, infections may play a substantial role. For example, one study, by Roberto Romero, the head of the Perinatology Research Branch of the National Institute of Child Health and Human Development, suggests that bacterial or other infections of the uterus cause 40% of premature births.

The infection paradigm. The invading bacteria initiate premature labor, McGregor says, by causing rupture of the amniochorion, the membrane sac that encloses the developing fetus within the uterus. They do so by producing protein-degrading enzymes called proteases that eat through the sac. The bacteria may also release phospholipases, fat-digesting enzymes that stimulate the production of the labor-inducing hormone prostaglandin.

If infections are an important cause of premature births, then their prevention or treatment might be an effective way of allowing pregnancies to reach full term. And in fact 3 years ago McGregor and his colleagues reported results from a clinical trial that suggest such a strategy is promising. The researchers gave either the antibiotic clindamycin or a placebo to each of 103 women who had been hospitalized with preterm labor. (Neither the doctors nor the patients knew who was getting the drug.) In a similar trial, they gave the antibiotic erythromycin or a placebo to each of 55 women who had already experienced premature rupture of the amniotic membrane (PROM).



SOURCE: ROBERTO ROMERO

Infectious invasion. Some researchers contend that infection by microbes *(dark spots)* triggers a cascade of events that ruptures the amniotic sac, bringing on early labor.

In both trials, the antibiotic treatment prolonged the pregnancies. The women who received clindamycin didn't give birth for an average of 35 days, while those in the control group had their babies after only 20 days on average. In the erythromycin trial, the pregnancy extensions were much shorter because the women's membranes had already ruptured, but even there the antibiotic seemed to help: The pregnancies of the treated group lasted an average of 13 days longer compared to an average of 4 days longer for the controls. As a result of the lengthening of the pregnancies, the newborns of the antibiotic-treated women, while still premature, tended to be larger and healthier and typically required less time in the nursery.

Despite these encouraging results, there's a problem with treating uterine infections with antibiotics. Such infections are usually well established long before an elevated temperature or white blood cell count brings them to medical attention. So, Romero says, researchers would like to identify the infections earlier, before they cause PROM or premature labor. McGregor feels that a tip-off may be an infection lower down in the reproductive tract—in the vagina, for example—because the offending micro-organisms characteristically start low and ascend. This view received some support in a clinical trial involving 1260 women (in press in the *American Journal of Obstetrics and Gynecology*), in which McGregor found that by identifying and systematically treating lower reproductive tract infections, it was possible to halve the rate of preterm PROM and preterm birth in women with bacterial infections. These dramatic results suggest a simple strategy. Says McGregor, "Systematically examine pregnant women to see if there are any of these factors, and if there are, treat them early and often."

No simple solution. Not everyone is convinced, however, that such a simple answer will suffice. Veteran perinatal researcher Paul MacDonald of the University of Texas Southwest Medical School in Dallas argues that, contrary to McGregor and Romero's conclusions, infection is not a cause of premature labor, but an effect. "The data assembled by us and Roberto are virtually identical—the interpretation is 180 degrees apart," MacDonald declares. "We hold the view that infection occurs after labor begins." MacDonald contends that the dilation of the cervix instigated by contractions permits microorganisms greater access to the birth canal.

In part, the opposing conclusions about whether the contractions or infections come first may be related to difficulty in pinpointing the exact start of contractions, as this is a somewhat subjective event. But while this debate continues, other scientists are sidestepping the infection-contraction question and searching for other early warning signs.

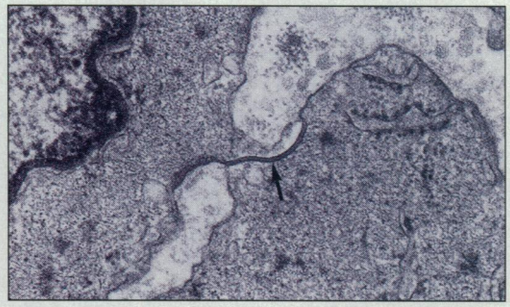
One such signal may be the hormone oxytocin, which helps initiate labor by locking onto specific receptors on the uterine muscles, thereby causing them to contract. That makes the hormone an obvious target for reproductive physiologist Laird Wilson of the University of Illinois, Chicago, and his colleague, peptide chemist George Flouret, who are testing an oxytocin antagonist—that is, a synthetic copycat protein that itself occupies the receptor, elbowing out the real thing—in baboons. "There's no question that the oxytocin antagonist will knock out the contractions—it's really good at that," Wilson says. They plan to begin clinical trials in humans in 1995.

Other oxytocin antagonists, although less potent than the one Wilson and Flouret are developing, have already shown promise in clinical trials. In the February 1994 issue of the *American Journal of Obstetrics and Gynecology*, for example, researchers from Ortho Pharmaceuticals in New Jersey reported results from a clinical trial of an antagonist called atosiban. They tested 112 women, all of whom were between 20 and 36 weeks pregnant and who had experienced preterm labor but not cervical dilation or PROM. A 2-hour intravenous infusion of atosiban decreased the women's contractions, although whether that decrease will translate into a prolonged pregnancy is not yet known. Those trials are continuing.

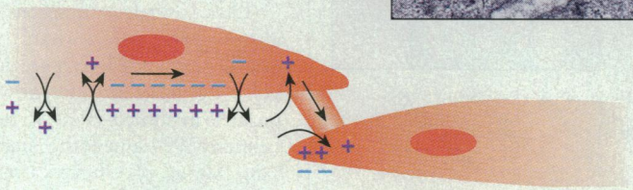
Looking for earlier warnings. While oxytocin appears to mark the very beginning of contractions, other scientists are searching for even earlier warning signs. "What we need is some factor that'll key us in to the fact that contractions are on the way," says Wilson. A protein known as fetal fibronectin may be one, says Mount Sinai's Lockwood, who notes that the protein serves as the glue that holds the amniochorion to the surrounding uterine wall. Then when the amniochorion separates from the uterus during labor and delivery, fibronectin is released into the cervix and vagina. Indications that fibronectin might be used to predict premature labor came in a study in which Lockwood

found that the protein was not detectable between 21 and 37 weeks of gestation, when most premature births occur, in vaginal fluids from women having normal pregnancies. But in women having premature contractions it showed up in high concentrations 82 percent of the time and—not surprisingly—was present in 94 percent of those whose membranes had already ruptured. "We found that it's a great marker for PROM, and also a pretty good marker for preterm delivery," says Lockwood.

The question now is whether fibronectin shows up early enough to be used as a marker. Adeza Biomedical in Sunnyvale, California, intends to find out. The biotechnology company has patented the protein as a marker and has applied to the Food and Drug Administration (FDA) for approval to use it as a predictor of prematurity. In early 1993 they embarked on a multicenter clinical trial in which a group of pregnant women at risk of delivering prematurely will be tested for fibronectin to see if it is later associated with premature birth. Lockwood and others are planning trials that will consider the presence of fibronectin the go-ahead to try pregnancy-prolonging antibiotic therapy.



ROBERT GARFIELD



Contraction connections. Gap junctions *(above)*, structures that serve to pass electrical signals between muscle cells *(left)*, allow labor contractions; detecting their increased presence might warn of premature labor.

Endocrinologist Jerome Strauss of the University of Pennsylvania School of Medicine in Philadelphia has recently found another possibly useful marker, an enzyme called matrix metalloproteinase-9, or MMP-9. Strauss and his colleague, Felipe Vadillo-Ortega, discovered that MMP-9, an enzyme that degrades collagen, is increased in fetal membranes during labor. With collagen so prominent in the makeup of the amniochorion and uterine wall, Strauss thinks, the buildup of MMP-9 may reflect a necessary degradation of those surfaces, so that the separation can be clean and safe. If he's right, then MMP-9 would not only be a potential marker, but it might also be possible to use inhibitors of the enzyme to prolong pregnancy. "There are known endogenous inhibitors of MMPs called tissue inhibitors of matrix metalloproteinases, or TIMPs," he says. "It might be possible to apply TIMPs as a way of inhibiting the function of the enzyme and therefore preventing either labor or preterm rupture of membranes."

Lockwood finds the approach promising. But he offers a major caveat: "You want to find a marker that's specific as possible to the fetus or fetal membranes. MMP-9 is getting there, but it's also made by the mother in other circumstances."

Another way of predicting premature labor may involve the substance nitric oxide along with gap junctions, which are membrane structures that serve as electrical connections between cells. Nitric oxide acts to relax smooth muscles, including those of the uterus, and reduced synthesis of nitric oxide by the uterus may help bring on the contractions of labor, says Texas' Garfield. Further evidence for this possibility comes from perinatal researcher James Roberts of the University of Pittsburgh, who has shown that in rabbits the enzyme that makes nitric oxide drops during labor to one tenth its concentration beforehand. In humans the difference isn't so dramatic, but the same pattern prevails. "There's also a decrease in the uterus's response to nitric oxide, so that it no longer inhibits uterine muscles to the same extent that it does during other times in pregnancy. There's a downregulation of the whole system," Garfield says.

Filling in the gaps. At the same time nitric oxide concentrations go down in the uterus, the number of gap junctions goes up. These gap junctions may also serve to facilitate contractions by helping the uterine muscle cells contract in a coordinated manner. Garfield, who originally detected the gap junction increase 17 years ago, thinks it might be due to the drop-off in nitric oxide production, although he cautions that such a cause-and-effect relationship remains to be proven.

No matter their relation to nitric oxide, gap junctions might still serve as a marker for the readiness of the uterus to begin contractions. Garfield is working on a method to exploit that possibility. "By measuring electrical activity in the uterus, you can detect when gap junctions appear and when a woman is prepared to go into labor," he says. The process is similar to that involved in measuring electrical activity in the heart.

Garfield places electrodes on the abdominal surface which pick up electricity in the muscles of the uterus. Early in pregnancy that activity is irregular; later, when the ability of uterine muscles to conduct electrical signals has improved so contractions can begin, the bursts become strong and steady. "It's a completely noninvasive and accurate predictor," says Garfield. "We've thoroughly tested it in the lab in experimental animals, and now we're testing it in the clinic on women. We can predict the onset of labor about 24 hours ahead of time." Other researchers are intrigued but cautious. "It could be a really exciting approach, but it hasn't been proven yet," says Roberts.

If labor can be predicted this way, though, can it be prevented at this point? A recent clinical study suggests that it can. In the 28 May issue of *The Lancet*, Christoph Lees of King's College Hospital School of Medicine and Dentistry in London described an experiment in which 13 women in the midst of preterm labor were treated with patches of nitroglycerine, which releases nitric oxide into the bloodstream. In every case labor was inhibited and pregnancy prolonged, by an average of 34 days.

But the day of diagnosis and treatment is not right around the corner, Garfield warns. "We have to be cautious about nitroglycerine therapy—that study was based on just 13 patients, and we need to try many more," he says. "And the electrical monitoring will be a very powerful technique, but it too needs to be further developed. But I've been working in this field for a long time, and I can tell you one thing: These are very exciting advances."

Others, too, feel the excitement is not overly premature. "Over the last 5, 10 years there has been a great improvement in our understanding of the underlying pathogenesis of preterm birth," says Lockwood. "And as we better and better refine our approaches, we're going to come up with therapies. My guess is that in the next 5 to 10 years we'll be testing new, effective ways to stop preterm birth in humans."

–Peter Radetsky

Peter Radetsky teaches in the science communication program at the University of California, Santa Cruz.

Additional Reading

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