NEWS

Quirks of Fetal Environment Felt Decades Later

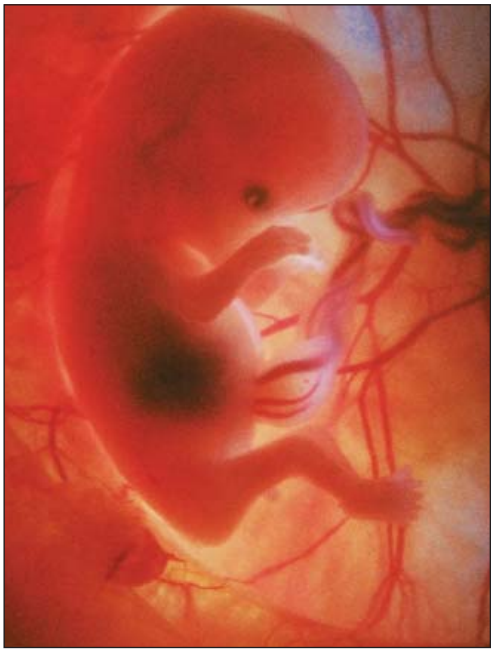
Normal babies who might have encountered adversity in the womb grow up to have a higher incidence of chronic diseases. Researchers are starting to figure out why

A fertilized egg has hoop after hoop to jump through during its upcoming 9 months of gestation. "We pass more biological milestones before we are born than we'll ever pass at any other point in our lives," says Peter Nathanielsz, director of the laboratory for pregnancy and newborn research at Cornell University in Ithaca, New York. Scientists have long believed that these milestones are molded by the environment an embryo, and later a fetus, encounters in the womb. Without enough folate, a fetus can't build and seal a backbone, for instance; a deluge of alcohol can interfere with delicate nervous system wiring; and certain medications taken by the mother might inhibit limb growth in the fetus she carries.

But some effects of gestational molding might lay dormant for decades before rearing their heads. A series of epidemiological studies began pointing to this conclusion in the late 1980s. Unexpectedly, adults who had been born with normal but low birth weights were found to have a higher risk of a constellation of adult diseases, including heart disease and type II diabetes. It was a startling idea. For years researchers have known that low birth weight, often associated with prematurity, is linked to a range of problems at birth, such as respiratory infections. But the new studies argued that low but normal birth weight—even in full-term, healthy babies—is a marker for susceptibility to diseases that show up 50 years later. And the list of adult ailments in which low birth weight has been implicated continues to grow: A May report in *The Journal of the American Medical Association* linked a woman's birth weight to her risk of developing gestational diabetes.

At first, researchers were skeptical. "There has been a kind of seeing what you want to see" in the epidemiological data, says Nigel Paneth, a pediatrician and perinatal epidemiologist at Michigan State University, East Lansing. Still, the theory that fetal environment influences adult disease susceptibility has been gaining legitimacy, particularly as it moves into the molecular biology arena. Indeed, some of the early skeptics are now pressing hard in animal studies to find out what, precisely, is behind the link between low birth weight and susceptibility to adult diseases.

At best, birth weight is a crude measure of fetal growth, and most believe that differences in birth weight, which rarely span more than 2 kilograms in full-term infants, are simply a rough marker for conditions encountered in the womb. A growing number of researchers are examining variables such as maternal stress, placental development, and embryo implantation that might underlie the perplexing find. Poorly formed kidneys, for example, might falter at regulating blood pressure. But for the most part, the mechanisms by which the fetal experience contributes to adult disease remain enigmatic.



Taking shape. Conditions in the womb appear to have an impact on risk of disease in adulthood.

Few had even considered such a delayed effect when British epidemiologist David Barker of the University of Southampton gave biologists a jolt in the late 1980s. After poring over birth and death records of thousands of people born in Hertfordshire, U.K., during the first third of the 20th century, he determined that babies weighing less at birth were likelier to die of heart disease. His statistical analysis of birth and death certificates revealed that 2.3-kg babies had double the rate of death from coronary heart disease of 4.5-kg babies. He later added hypertension, stroke, and type II diabetes to the list. Barker hypothesized that maternal malnutrition was largely responsible.

"I was skeptical," recalls Janet Rich-Edwards, an epidemiologist at Harvard Medical School in Boston, about her first encounters with the so-called Barker hypothesis. She wondered about all the data Barker's analysis couldn't capture because his subjects weren't available for interviews: socioeconomic status, family history, smoking habits. But Rich-Edwards had her own extraordinary, living cohort on her hands: 121,700 nurses who make up the Nurses' Health Study, a group that continues to supply scientists with valuable medical data. In 1992, the team added a question about birth weight to the routine questionnaire the volunteers filled out.

"I was sure I was going to be able to adjust away this association between birth weight and later disease" by taking into account other factors, says Rich-Edwards. "In fact I couldn't. … My own data convinced me." Her group found that a woman who weighed between 2.3 and 2.5 kg at birth—low, but within the normal range—had a 23% higher risk of heart disease and an 80% higher risk of type II diabetes than someone who weighed between 3.2 and 3.9 kg. These epidemiologists have also found that babies weighing about 2.5 kg at birth had about half the chance of developing breast cancer as adults, compared with those in the 4-kg range—suggesting that there's no straightforward relationship between low birth weight and susceptibility to disease.

The risks of maternal malnourishment were reinforced by a study of Dutch adults conceived or born during late 1944 and early 1945, when the Nazi army halted food transport to occupied areas of the Netherlands. Food consumption plunged from 1800 calories a day to as little as 400. Tessa Roseboom, now an epidemiologist at the University of Amsterdam, and her colleagues found that babies conceived while their mothers were severely malnourished tended to share the same problems in adulthood as the people in Barker's study. Many of her volunteers didn't have low birth weights, however, suggesting that a troubled environment before birth might leave no easily measurable trace behind.

Food and stress

To uncover the molecular actions that can go awry early on and prompt disease decades later, researchers are reaching beyond the risk factors first identified by the fetal origins field, such as the baby's weight or length. Simon Langley-Evans, a nutritionist at the University of Nottingham, U.K., experimented with feeding pregnant rats a low-protein diet. Normally their diet would be upped to 12% protein during pregnancy, but Langley-Evans gave them 9%—what they

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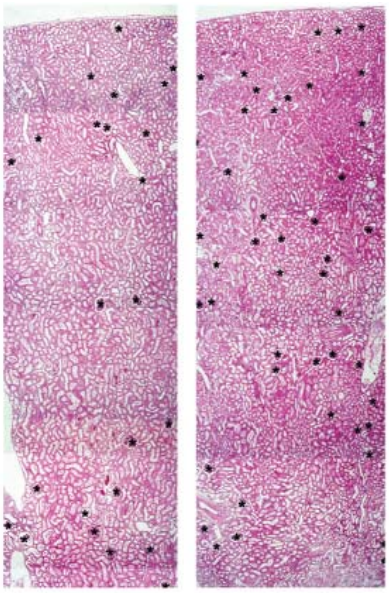
would consume if they weren't pregnant. As seen in previous studies, the animals' offspring grew up to have high blood pressure.

But Langley-Evans had a hunch that low protein wasn't directly responsible for the hypertension. Rather, he hypothesized, it was blocking a critical enzyme in the placenta that keeps stress hormones in the mother's blood from reaching the fetus. To test this, he gave pregnant rats a drug that stopped them from producing stress hormones. The offspring of these rats, fed low-protein diets, then had normal blood pressure—suggesting that the hormones, not the diet, were behind the hypertension. Furthermore, when pregnant rats on high-protein diets were given a drug that inhibited the placental enzyme, their offspring had high blood pressure, just as if their mothers had ingested too little protein. Says Langley-Evans: "My experience is any manipulation of the diet has pretty much the same effect"—inhibiting the enzyme, which goes by the unwieldy name of 11β-hydroxysteroid dehydrogenase (type 2), and allowing more stress hormones into the fetus's bloodstream.

Even well-fed pregnant animals can bathe a fetus with excess stress hormones if they are under significant stress. Marelyn Wintour, a fetal physiologist at the University of Melbourne, Australia, found that injecting the stress hormone cortisol into pregnant sheep gives their offspring high blood pressure for years. She presented her research at the Federation of American Societies for Experimental Biology conference in April.

Endocrinologist Jonathan Seckl of Western General Hospital in Edinburgh, U.K., believes that excess levels of stress hormones in the fetus "reset" a major mediator of stress in the body, the hypothalamic-pituitary-adrenal (HPA) axis, making it hypersensitive to even banal events. In other words, the body churns out glucose, cortisol, and other stress-related substances when they wouldn't normally be needed—a result now observed in rats, mice, sheep, and low–birth-weight humans. Elevated glucose levels could help explain the high rates of type II diabetes found in those whose mothers were under stress before birth, such researchers claim.



Underdeveloped. Pigs lacking adequate protein during gestation are born with fewer nephrons (black dots).

Stress hormones like cortisol are also known to be powerful regulators of gene expression. Researchers including Harvard pediatric nephrologist Julie Ingelfinger say these hormones might well turn on and off genes critical to the fetal development of organs such as the kidney, further contributing to later disease.

Why don't these effects show up until well after birth? Varying theories seek to answer that question. Cornell's Nathanielsz suggests that the animals are born fighting against, say, high blood pressure, and at some point their defense mechanisms collapse. Langley-Evans says it's possible that the animals are born susceptible to certain diseases, and other factors that contribute to them, such as poor diet, can push those more at risk over the edge.

From the outside in

One puzzle researchers face is that low–birth-weight babies aren't all born to anxious mothers subsisting on bread and water. Many perfectly healthy women give birth to infants whose growth is somehow retarded in the womb, a reminder that it's not only the mother's condition that matters, but what reaches her fetus through the placenta.

Remarkably little is known about placental development and defects. In humans, a fertilized egg has about a week after fertilization to implant itself in the walls of the uterus. Implantation reshapes uterine arteries so they can better deliver nutrients and oxygen. As the fetus grows, the placenta becomes larger and thinner—a process called remodeling—to sustain its charge.

Although drastic defects in the placenta often lead to miscarriage, more subtle problems seem to cramp development of fetal organs, particularly the kidneys and heart. "If the placenta is poorly formed and does not remodel properly, this puts cardiovascular stress on the fetus," says Kent Thornburg, director of the heart research center at Oregon Health & Science University (OHSU) in Portland. Thornburg's group is currently testing a hypothesis that improper placental remodeling in the latter half of pregnancy contributes to heart disease in adult sheep.

In another OHSU lab, nephrologist Susan Bagby is using pigs to explore how stunted kidney development might, along with an overactive HPA axis, contribute to high blood pressure later on. Because kidneys control the body's balance of salt and water, they are a crucial moderator of blood pressure. Furthermore, the functional units of the kidney, called nephrons, all form before birth in humans. "The number of nephrons that you have is very important in determining the capacity of the kidney to deal with the world," says Bagby. Langley-Evans has found that low-protein diets during pregnancy cut the number of nephrons in rat offspring by a third. Researchers are only beginning to consider whether some kidney failure in adult humans might have its roots in the womb.

High-strung. Stressed-out ewes give birth to lambs with high blood pressure.

Even more mysterious than placental development is what comes before it: implantation. Richard Schultz, a developmental and reproductive biologist at the University of Pennsylvania in Philadelphia, has found that variations in artificial embryo culture can dramatically alter gene expression.

The true start, of course, comes not with implantation but with the genes endowed during the combination of egg and sperm. Fetal origins research, which began with both feet firmly planted in the environmental camp, has struggled with how big a role genes might play. Birth size, for example, is probably partly genetic. Researchers suspect that the kind of "smallness" that puts babies at risk of later disease is not genetic. At-risk babies, the theory goes, were destined to be larger but suffered retarded growth in the womb. They can be distinguished from genetically small infants, many in the field say, by their proportionally large heads. A popular theory holds that some form of stress before birth prompted these fetuses to restrict their body's growth in order to protect their brain.

Ultimately, most agree that genetic and environmental influences are braided together. There might be genetic variations in the fetus or mother that, say, prevent the placen-

ta from properly remodeling or make it more likely to admit excess cortisol.

Sculpting destiny

If the 9 months spent in the womb help shape susceptibility to disease, what, if anything, can be done to reverse or even prevent ill effects? Given how little is known about critical periods in pregnancy when a given stressor might produce a given defect, the likelihood of preventing problems before they start remains remote.

Reversing these problems could prove slightly more feasible. One possibility might be halting a common phenomenon among babies born smaller than intended, called catch-up growth. These infants born at, say, the 20th percentile for growth hit the 80th percentile by the time they're school age. Some theorize that this occurs because a baby conditioned in the womb to anticipate fewer nutrients gains more from each gram of food it consumes. Studies in humans and animals suggest that catch-up growth makes adult diseases associated with low birth weight much likelier. Bagby is experimenting in her pigs to see whether inhibiting catch-up growth—which, in humans, might be easier said than done—will preserve kidney function and normal blood pressure in adults.

In another approach, treating newborn rats between 2 and 4 weeks after birth with drugs to counteract high blood pressure, and then withdrawing the treatment, has also been shown to permanently reverse the effects of low protein prior to birth, says Langley-Evans.

Still, researchers say they're a long way from addressing the implications of troubled fetal environments in the clinic, especially because low birth weight remains the only simple measure of susceptibility to later disease. Rebecca Simmons, a neonatologist at the University of Pennsylvania and Children's Hospital of Philadelphia, routinely treats low–birth-weight babies. But at a loss to quantify risks, she rarely volunteers information on the likelihood of later disease with parents. "The reason we're not talking about it with the parents now," she says, "is that we don't know what to do."

–JENNIFER COUZIN

NEWS

Cells Exchanged During Pregnancy Live On

Microchimerism, viewed at first as an oddity, has been linked to autoimmune diseases and complications of pregnancy

A mother's love is enduring. But most mothers would be surprised to discover that there's a similarly enduring physical bond: Cells from a fetus can live on in the mother's body for decades after pregnancy, a situation called microchimerism. Likewise, a mother's cells can also survive for many years in her child.

When this phenomenon was first reported in the mid-1990s, scientists scoffed at the notion that these cells could persist for so long, tolerated by their host's immune system. "Everyone said it can't be true," says rheumatologist Michael Lockshin, director of the Barbara Volcker Center for Women and Rheumatic Disease at the Hospital for Special Surgery in New York City. "But now everyone who looks finds it."

In some cases, the cells might be benign guests: self-perpetuating lines of stem cells that can reproduce and even give rise to other types of cells, all without harming their host. But a growing body of research, still preliminary, suggests that the cells might also be at the root of some autoimmune diseases and other conditions.

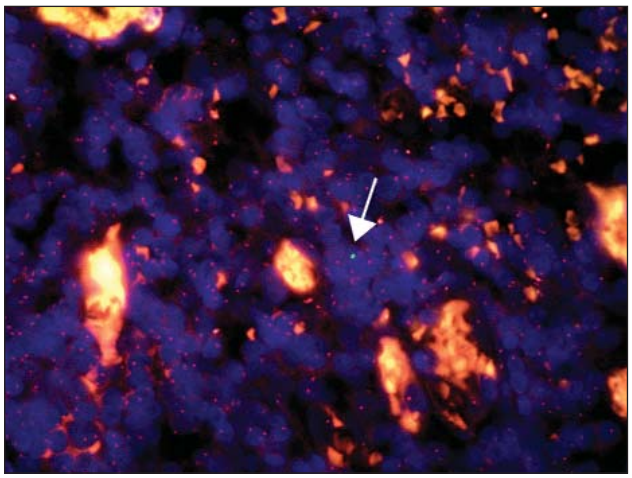
Indeed, microchimerism might help explain one of the puzzles about autoimmune diseases: why many of them strike more women than men. No one knows how many women carry foreign cells around from past pregnancies, but several studies have shown that women with certain autoimmune diseases are more likely to harbor such cells than healthy women. "When you see that this is a real phenomenon, it gives you a different perspective," says pediatric hematologist William Reed of the Children's Hospital Research Institute in Oakland, California. "You begin to ask yourself whether a disease might have a pathogenesis that you've never considered before."

And it's not only the long-lived cells that might be making mischief. Reproductive biologists have known for some time that fetal cells course through the bloodstream of pregnant women, but in the past 4 years researchers have discovered that this temporary invasion might be implicated in two common complications of pregnancy.

Inner turmoil

Fetal microchimerism was uncovered quite by chance. In 1992, medical geneticist Diana Bianchi, then at Children's Hospital in Boston, was trying to develop a method for prenatal diagnosis that relied on isolating fetal cells from the blood of pregnant women. Her team was separating out cells that carried a protein known as CD34—a marker for so-called hematopoietic stem cells that give rise to cells of the immune system—based on a hunch that CD34 would be a good marker for fetal cells.

Blood from 13 of the pregnant women they studied contained CD34-positive cells with a Y chromosome, indicating that the fetuses from which the cells came were male. But amniocentesis showed that only nine of those women were carrying male fetuses. "We were mystified," says Bianchi, who is now at Tufts–New England Medical Center in Boston. They checked to see whether any of the four women with unexplained male cells had other children who were male, and two of them did. The other two had previously terminated pregnancies in which the sex of the fetus was not known. "That is when the hypothesis began to take shape," says Bianchi.



Under mom's skin. A cell with a green-stained Y chromosome, presumably from a son, was found in a skin biopsy from a woman with systematic sclerosis.

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To test the idea that fetal cells from a past pregnancy can linger, Bianchi and her colleagues examined the blood of mothers who were not pregnant. They chose eight mothers of boys, because testing for the presence of a Y chromosome could easily distinguish the sons' cells from their mothers'. Six of the women, including one whose youngest son was 27 years old, had male cells still circulating in their blood. The idea was so surprising that it met resistance. "I lost count of how many times this paper was rejected," Bianchi told the