New Role for Estrogen in Cancer?

Although estrogen was supposed to act mainly as a growth factor in promoting cancers, new work suggests that products it forms in the body may also cause the initiating mutations

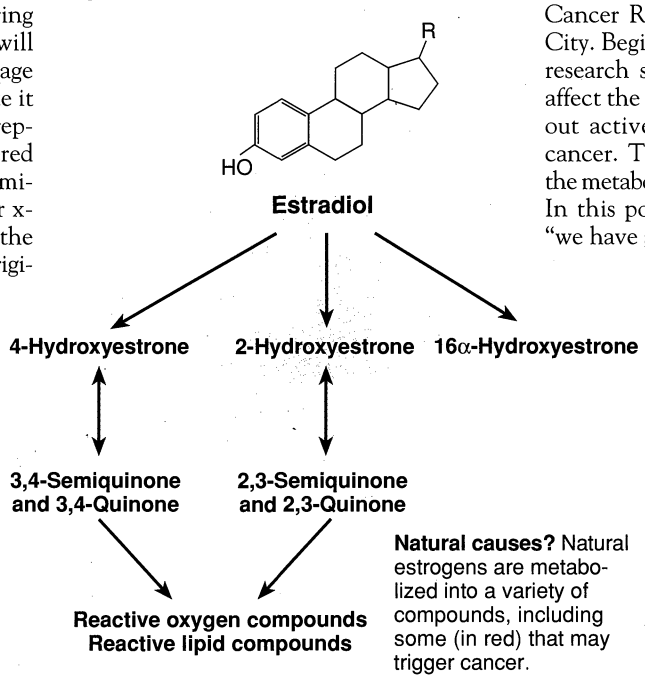
The link between the female hormone estrogen and cancer is hard to miss. Both epidemiological and cell biology studies have indicated that it contributes to the development of three of the top five cancers of women—those of the breast, uterus, and ovaries—which together account for an estimated 240,000 new cancer cases a year in the United States alone. Now there is new evidence that estrogen's involvement in these cancers may be much deeper than was thought.

Researchers have long known that the hormone is a powerful stimulator of cell proliferation. The common belief has been that cell growth promotes cancer development by increasing the chances that a cell bearing a potentially cancer-causing mutation will multiply. As for the initial genetic damage itself, researchers have tended to attribute it either to spontaneous mistakes in DNA replication as cells divide or to damage triggered by external sources: environmental chemicals, such as those in cigarette smoke, or x-rays and other forms of radiation. But the new evidence points to another culprit originally fingered as a suspect years ago: the metabolic byproducts formed by estrogen in the body.

Cell culture studies show, for example, that estrogen metabolites can bind to DNA and trigger damage. The same compounds also produce cancer in lab animals. And recent epidemiological work suggests that women who have reduced amounts of the enzymes that help sop up those reactive estrogen byproducts are at higher risk for developing breast cancer. Taken together, these studies provide "pretty good data that, by themselves, hormones can be complete carcinogens," says David Longfellow, a chemist with the National Cancer Institute in Bethesda, Maryland, who is organizing a conference on the topic, set to take place next week.* If the data hold up, they could force researchers to take a closer look at the role of hormones in the body, and ultimately offer new avenues for cancer prevention by removing potentially damaging compounds before they build up.

But there is likely to be plenty of debate at Longfellow's conference. The view that hormones initiate as well as promote cancer remains far from universal. "This has been hugely controversial," says Craig Jordon, a pharmacologist at Northwestern University Medical School in Evanston, Illinois. "It's an interesting possibility," adds Jan-Ake Gustafsson, an estrogen metabolism expert at the Karolinska Institute in Huddinge, Sweden. "But the hard data are still lacking."

Disputes rage within the ranks of true believers as well. Estrogen produces several metabolites, and competing research teams—each backing its own horse in the race—argue for different metabolites as being the most important gene disrupter. "It's a mess," says Patricia Thompson, a molecular biologist at the National Center for Toxicological Research (NCTR) in Jefferson, Arkansas. "But it's a very hot area, and some people feel very strongly about what they think is going on."



Natural causes? Natural estrogens are metabolized into a variety of compounds, including some (in red) that may trigger cancer.

Starting from scratch

As early as 20 years ago, researchers recognized that estrogen's growth-stimulating effects could make it a cancer promoter. Rats, for example, more often develop mammary tumors if they are subjected to both a chemical carcinogen and estrogen than to either alone. That picture also fits with epidemiology studies showing that women who are exposed to estrogens for longer—either through early onset of menstruation or late menopause—have an increased risk for developing breast cancer.

Proponents of the estrogen metabolite hypothesis don't doubt that estrogen promotes cancer growth. Those effects "are clearly very important," says James Yager, a toxicologist at the Johns Hopkins University School of Hygiene and Public Health in Baltimore. But when it comes to understanding just what causes the initial DNA damage, "the conventional model is just hand waving," says Leon Bradlow, an endocrinologist and estrogen metabolite hypothesis supporter at the Strang Cancer Research Laboratory in New York City. Beginning in the mid-1970s, however, research showed that estrogen metabolites affect the levels of enzymes designed to clear out active compounds that might initiate cancer. That suggested another possibility: the metabolites themselves might be involved. In this possible mechanism, says Bradlow, "we have a more cogent case."

The suspects

Just what that mechanism is depends on who you listen to, however. The list of possible suspects is long. For example, the principal natural estrogen, estradiol, is transformed in cells into three main components: 2-hydroxyestrone (2-HE), 4-hydroxyestrone (4-HE), and 16-α-hydroxyestrone (16-α). These can react directly with DNA or be metabolized further into other reactive compounds.

Most researchers agree that 2-HE isn't dangerous, because it fails to show up as a mutagen in cell culture studies or as a carcinogen in animals. Instead, proponents of the metabolite hypothesis fall into two main camps, one blaming 4-HE and the other 16-α.

For members of the first camp—led by Joachim Liehr of the University of Texas Medical Branch in Galveston and Ercole Cavalieri of the University of Nebraska Medical Center's Eppley Institute for Research in Cancer and Allied Diseases—one touchstone is a 1986 experiment Liehr and his colleagues performed with male Syrian golden hamsters. These animals are unusual in that their kidneys express the receptors through which estrogens turn on cell growth.

* "Estrogens as Endogenous Carcinogens in the Breast and Prostate," Westfields International Conference Center, Chantilly, Virginia, 16–17 March.

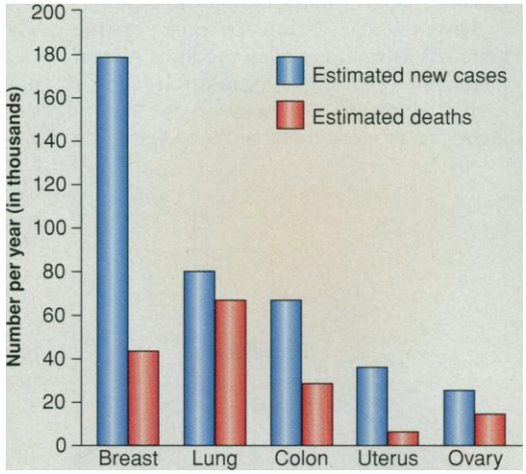
When the researchers exposed the hamsters to a synthetic estrogen that is an even more potent activator of the receptor than the natural hormone, relatively few of the animals developed kidney cancers. But nearly all hamsters given 4-HE, which still binds to the estrogen receptor but is a less active growth stimulator, developed tumors within 6 months. "That tells me receptor-mediated binding cannot be the sole process responsible for tumor induction," says Liehr.

Later studies have shown that 4-HE is produced at the scene of the crime—the tissues where estrogen-linked cancers develop. Most estrogen metabolism takes place in the liver, where the product is primarily the safe 2-HE. But in 1996, researchers led by Johns Hopkins University environmental health scientist Tom Sutter discovered that an enzyme known as cytochrome P-4501B1 converts 17-β-estradiol to 4-HE. And last month, they reported in *Carcinogenesis* that this enzyme is more abundant in the breast than in tissues not prone to estrogen-linked cancers. Sutter adds that the enzyme also seems to be present in other cancer target tissues as well, including the uterus and ovaries. Indeed, human breast tumor tissue has an even higher level of 4-HE than normal tissue, perhaps because the enzyme is overactive there. But the data on whether the same is true for other human tumor tissues are still out.

What actually damages DNA and leads to tumor development, says Cavalieri, may be the products of 4-HE. Numerous enzymes can change 4-HE into compounds called 3,4-semiquinones and 3,4-quinones. These compounds, Cavalieri and his colleagues report in the 30 September 1997 issue of the *Proceedings of the National Academy of Sciences*, bind to DNA, creating adducts, both in the test tube and in living animals, but then quickly fall off, taking with them two of DNA's bases, adenine and guanine. These gaps in the DNA, Cavalieri and his colleagues believe, have a strong potential to create gene mutations. "We believe it's [these] adducts that cause cancer," says Cavalieri. Indeed, when he and his colleagues injected 4-HE quinones into newborn mice, the result was liver cancer.

Two recent epidemiology studies seem to support the link between 4-HE and its byproducts and cancer. These studies focused on an enzyme known as catechol-O-methyltransferase, or COMT, that seeks out 2-HE and 4-HE and tags on a methyl group, making them more water soluble and therefore easier for the body to excrete. People who make less of the enzyme than others would in theory clear 4-HE and its toxic brethren more slowly. So the two studies—one led by Yager, the other by Thompson and her NCTR colleague Christine Ambrosone—looked to see if low levels of the enzyme would put women at greater risk of beast cancer.

Both studies, each of which included between 230 and 300 women, did find that particular groups of women with low COMT levels had a higher incidence of breast cancer. The picture isn't altogether tidy, though. Yager's study, published in the 15 December 1997 issue of *Cancer Research*, found that only postmenopausal women with low COMT have a higher risk for breast cancer. In contrast, Thompson and Ambrosone's study, which is not yet published, saw a higher risk only in premenopausal women who smoke. Still, says Richard Weinshilboum, a pharmacogeneticist and metabolism expert at the Mayo Medical School in Rochester, Minnesota, the results "begin to make you think there is some fire under this smoke."



SOURCE: THE AMERICAN CANCER SOCIETY

Alarming numbers. Estrogens are linked to three of the most common cancers in U.S. women.

Take two

Bradlow, however, sees 4-HE and its brethren as bit players. "In certain animal systems it is important," says Bradlow. "But it's a very minor product in humans. We don't think it plays a major role" in cancer development. The problem, he says, is that in humans only about 5% of estrogens in the tissues end up as 4-HE. More than twice as much end up as 16-α, which he thinks is the most dangerous estrogen metabolite.

Bradlow offers multiple lines of evidence to support this idea. He and his Strang colleague Nitan Telang found that cultured breast cells subjected to 16-α have an increased rate of DNA repair activity, an indication that the cells have a higher than normal level of mutation. In addition, in work that has not yet been published, Bradlow and his colleagues have seen that in tissue stains, 16-α invariably shows up in and around breast tumors.

He also cites human epidemiological studies. One, which he and his colleagues are about to publish in the *British Journal of Cancer*, followed 85 women. It showed that those who had lower 2-HE to 16-α ratios in their urine were more likely to go on to develop breast cancer than were women in whom that ratio was weighted more strongly in favor of 2-HE.

Even as the two camps continue to amass data to seal the case against their suspects, other possible culprits are continuing to emerge as well. Metabolites from both 4-HE and 2-HE can go on to create other reactive products, such as superoxide, hydroxyl radicals, and partially oxidized and reactive lipids, all of which themselves could be involved in damaging DNA and turning cells cancerous. Meanwhile, the same link between hormones and cancer is also being investigated as a cause of prostate cancer, although for now the data here remain more shallow. Of course, even though separate teams are currently backing their own favorite molecules, there's a good chance that everybody is in part correct, each feeling a different portion of the elephant. "It's not necessarily this or that, but this and that," says Yager.

The skeptics

But many estrogen researchers aren't ready to back any metabolite hypothesis and remain troubled by what they see as inconsistencies. For one, estrogens and their metabolites don't register as highly mutagenic on standard tests such as the Ames test. "You test estrogen in a straightforward assay and it simply comes out negative," says Gustafsson. Liehr counters that this is expected, because the Ames test and others like it are designed to measure potent rather than weak carcinogens. "If any endogenous compound was a strong carcinogen, we'd all be dead," says Liehr.

The critics also cite the fact that estrogen is present in the body in tiny quantities—too tiny, in fact, to produce enough byproducts to worry about, says Jonathan Li, a pharmacologist and cancer researcher at the University of Kansas Medical Center in Kansas City. Liehr agrees that the amounts of estrogen circulating in the blood are low, but points out that's not all there is to worry about. Estrogen is also synthesized directly in cells in target tissues by an enzyme called aromatase. It converts the androgen testosterone, which women make in small quantities, into estradiol.

Indeed, Richard Stanten and his colleagues at the University of Virginia Health Sciences Center in Charlottesville have found that the amount of estrogen synthesized by aromatase in breast tissue far exceeds the amount of the hormone circulating in blood.

"Since the tissue itself makes estrogen, there is enough present to make high levels of estrogen metabolism to make genotoxic activity plausible," says Stanten.

Plausible but not certain. Most estrogen metabolite researchers believe that certainty will come in time as the studies continue to roll in. Studies of mice that have been engineered to either over- or underexpress particular metabolite-controlling enzymes could be particularly enlightening, says Eppley Institute biochemist Eleanor Rogan. But even then, sorting out all the signals won't be easy because of the complexity of the system, she says.

But the effort will be worthwhile, because if further evidence does nail down the idea that estrogen metabolites are mutagenic, it may be possible to intervene to reduce the risk of cancer, says Longfellow. If it turns out that women who over- or underexpress crucial enzymes have an increased cancer risk, for example, researchers could try to design drugs to bolster or block the levels of these compounds. "After all, these are things that can be modulated," Longfellow says. But for now the primary challenge remains confirming the role of estrogen metabolites in the first place. "The evidence is building," says Yager. "But the burden of proof still lies in developing more direct evidence."

–Robert F. Service

Additional Reading

B. T. Zhu and A. H. Conney, "Functional role of estrogen metabolism in target cells: Review and perspectives," *Carcinogenesis* **19**, 1 (1998).

J. A. Lavigne *et al.*, "An association between the allele coding for a low activity variant of catechol-*O*-methyltransferase and the risk for breast cancer," *Cancer Research* **57**, 5493 (1997).

E. L. Cavalieri *et al.*, "Molecular origin of cancer: Catechol estrogen-3,4-quinones as endogenous tumor initiators," *Proceedings of the National Academy of Sciences* **94**, 10937 (1997).

J. Fischman *et al.*, "The role of estrogen in mammary carcinogenesis," *Annals of the New York Academy of Sciences* **768**, 91 (1995).

EVOLUTION

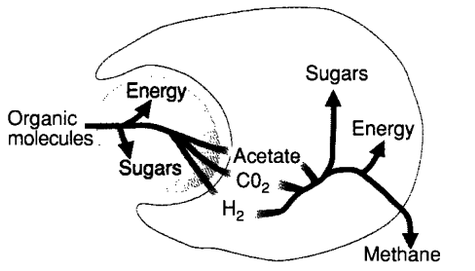
Did the First Complex Cell Eat Hydrogen?

Successful unions can start off in the strangest ways. Take eukaryotic cells, which compose all "higher" organisms and generally contain energy-producing organelles called mitochondria. Mitochondria were once free-living bacteria, and most researchers believe that early in evolution ancestral eukaryotic cells simply ate their future partners. But two researchers are now arguing for a less haphazard start to this ancient partnership. The first eukaryotes, they say, had an appetite for the waste products of the original mitochondria. The union of these organisms was simply a matter of survival.

In last week's *Nature*, William Martin of Braunschweig Technical University in Germany and Miklós Müller of Rockefeller University in New York City draw on genetic data, biochemistry, and the lifestyles of some simple organisms today to argue that the first eukaryote evolved from a methanogen, a microbe that consumes hydrogen and carbon dioxide and produces methane. Its partner—the future mitochondrion—was a bacterium that made hydrogen and carbon dioxide as waste products.

Scientists pondering how the first complex cell came together say the new idea could solve some nagging problems with the prevailing theory. "It's eminently sensible," says evolutionary biologist Russell Doolittle of the University of California, San Diego. But he and others aren't ready to embrace the new scenario. "It's elegantly argued," says Michael Gray of Dalhousie University in Halifax, Nova Scotia, but "there are an awful lot of things the hypothesis doesn't account for."

In the standard picture of eukaryote evolution, the mitochondrion was a lucky accident. First, the ancestral cell—probably an archaebacterium, recent genetic analyses suggest—acquired the ability to engulf and digest complex molecules. It began preying on its microbial companions. At some point, however, this predatory cell didn't fully digest its prey, and an even more successful cell resulted when an intended meal took up permanent residence and became the mitochondrion.



B. FINLEY/INST. OF FRESH WATER ECOLOGY

B. FINLEY

So happy together. Exchanges of molecules including hydrogen may have bound microbes together in the first complex cell. In a modern analog, bacteria snuggle close to hydrogen-producing organelles (dark structures, ~2 micrometers long) inside a protist *(left)*.

For years, scientists had thought they had examples of the direct descendants of those primitive eukaryotes: certain protists that lack mitochondria. But recent analysis of the genes in those organisms suggests that they, too, once carried mitochondria but lost them later (*Science*, 12 September 1997, p. 1604). These findings hint that eukaryotes might somehow have acquired their mitochondria before they had evolved the ability to engulf and digest other cells.

How it might have happened came to Martin one evening when he looked at a picture of a protist called *Plagiopyla*. These one-celled eukaryotes have hydrogen-producing organelles called hydrogenosomes, which are thought to be related to mitochondria. And in their cytoplasm, clustered among those organelles, live hydrogen-consuming methanogens.

Looking at those hungry methanogens, Martin recalls, "the cell sort of evolved before my eyes." He discussed the idea with Müller, and "all of a sudden everything fell into place," Martin says. They concluded that what they saw inside the protist—the partnership of the organelles and the methanogens—mirrored the union that had led to the first eukaryotic cell.

Müller and Martin think that the association between the ancestral methanogen and a hydrogen-producing bacterium started casually, in an oxygen-free, hydrogen-rich environment. The microbial pair later found itself far from that original environment, where the methanogen could not survive without its partner. Then, Martin and Müller suggest, a transfer of genes cemented the partnership, allowing the host to enclose its guest completely. The new genes enabled the methanogen to import small molecules, make sugars, and break them down into food for the enclosed cell. These genes probably came from the guest bacterium, which could also use oxygen to produce energy—as mitochondria do today.

The hypothesis is "the most cogent explanation for why a eubacterium and an archaebacterial cell should get together in the first place," says Gray. If it is right, cur-