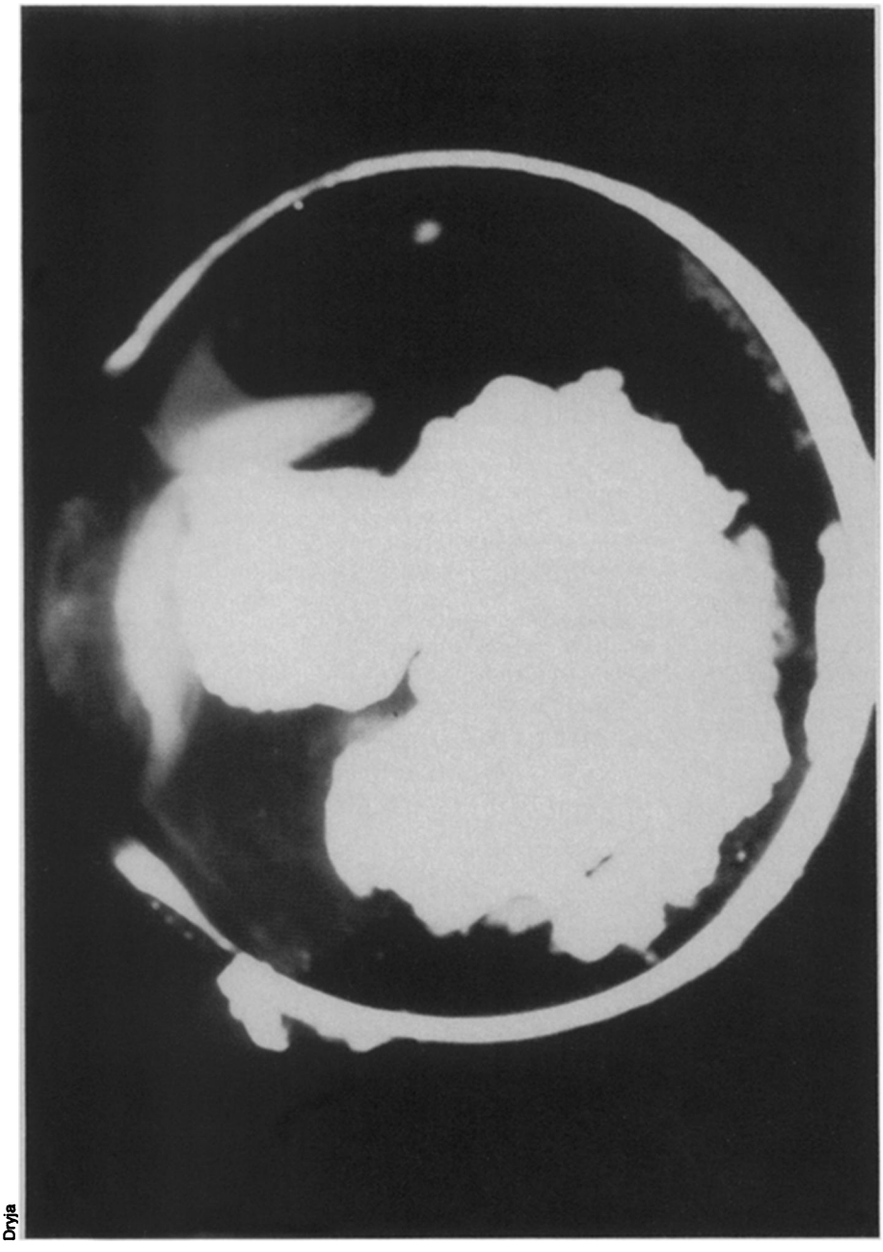
RETINOBLASTOMA: UNMASKING A CANCER

Recent studies suggest that the lack of a vital normal gene, rather than the presence of a cancer-causing oncogene, may play a key role in the development of some cancers



Dryja

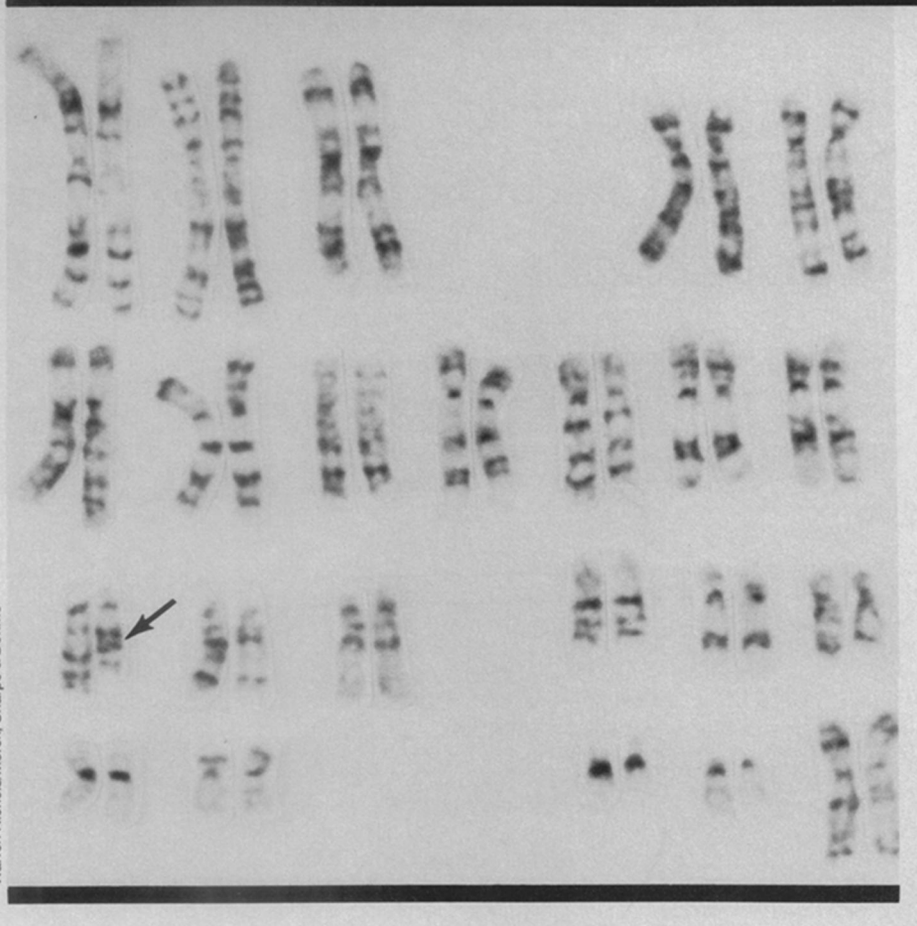
Cross section of an affected eye of a patient with retinoblastoma. The large white mass in the center is the tumor, flanked by the lens on the left and the retina on the right.

By STEVEN I. BENOWITZ

A major advancement in cancer research in recent years has been the discovery of oncogenes, or cancer-causing genes. First described in the 1970s by scientists at three New England laboratories, oncogenes are believed to be normal genes that for some reason go awry (SN: 9/26/81, p. 199). But at several laboratories across the country, researchers have begun to uncover startling new evidence that points to exactly the opposite. What may be important in some cancers, scientists now contend, is not the presence of an oncogene but the absence of a normal gene.

The prototype for such diseases is retinoblastoma, a rare, inherited childhood cancer of the eye. Children can get retinoblastoma in one of two ways: It can be inherited from an affected parent (50 percent of such a person's children will get it), the culprit gene residing in every cell of the body; or it can occur spontaneously, a mutation in an odd retinal cell. Both forms are equally uncommon, striking between 200 and 300 newborns and young children in the United States each year, and one in 18,000 worldwide.

Retinoblastoma was once a ruthless killer, attacking children at birth or soon after. From its starting point in the retina, the disease would migrate up the optic nerve to the brain; the young victim would



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Chromosomes from a blood cell of a retinoblastoma patient. Arrow points to the defect in the right chromosome 13 that allows expression of the mutant retinoblastoma gene.

die of brain cancer. But about 20 years ago, thanks to improved surgical techniques and better use of anticancer medicines and radiation therapy, this pattern was interrupted. Doctors learned to remove the affected eye or eyes and, when necessary, part of the optic nerve as well. In some cases, the disease can now be arrested with radiation alone. Today, 85 percent of children with retinoblastoma can expect to reach the five-year survival mark that doctors often equate with cure.

But what should have ended as a quiet victory in the battle against cancer didn't. Several years ago, researchers began to notice an alarming trend: Many of these "cured" retinoblastoma patients were showing up in clinics and doctors' offices with second cancers. These other cancers weren't remnants of retinoblastoma spread to other sites in the body. What appeared to be happening was that for the first time, a noticeable number of children with the inherited form of retinoblastoma were living longer and developing completely new diseases some 10 to 15 years later, in adolescence. Many of these were bone cancers called osteosarcomas.

"No one would have suspected that the retina would have anything to do with a totally different type of tissue like bone," says Webster Cavenee, associate professor of microbiology and molecular genetics at the University of Cincinnati Medical School. "But clearly there was some connection."

Scientists several years before had pinned the genetic blame for retinoblastoma on a region of DNA on the long arm of chromosome 13 (SN: 5/9/81, p. 297). They knew a defective gene was involved — a gene missing some genetic material — but the precise location of the gene, as well as how it worked, remained obscure. In 1971, while at M. D. Anderson Hospital and Tumor Institute in Houston, Alfred Knudson examined the cells of children of retinoblastoma victims and saw that some of the children had a chromosomal deletion but no cancer. "It was clear," says Knudson, now at the Institute for Cancer Research in Philadelphia, "that just having this mutant gene did not mean a person had to get cancer." Knudson proposed that some second step was needed for cancer to develop.

Using these clues as starting points, and enlisting the aid of sophisticated recombinant DNA techniques, Raymond White of the University of Utah in Salt Lake City, and Cavenee, who at the time was at the same university, looked at chromosome 13 DNA taken from hereditary retinoblastoma tumor cells. The researchers were searching for genetic differences between a patient's normal heterozygous blood cells and cancerous retinal cells.

Cavenee and White found two kinds of chromosome 13s in each blood cell: one normal and one abnormal, the latter conspicuously lacking a small portion of DNA. They expected that. What they didn't expect was that in each tumor cell, there was only *one* kind of chromosome 13 — the one with the telltale deletion — and no normal one. Moreover, in roughly half the cases they examined, there were two copies of the incomplete chromosome 13, and none of the normal. Tumor cells from the other retinoblastoma patients had only one abnormal chromosome 13.

At a genetics meeting sponsored by the March of Dimes last year at Jackson Laboratory in Bar Harbor, Maine, Cavenee and White concluded that if a child inherits a mutant chromosome 13 from a parent carrying the retinoblastoma gene and a normal chromosome from the other parent, the disease does not necessarily develop, verifying what Knudson had suggested more than a decade before. To create the cancer, the scientists hypothesized further, something else has to happen: The dominant normal gene must be lost. In any case, the normally masked recessive cancer-causing gene then takes over.

All conventional oncogenes are dominant — that is, whenever the activated oncogene is present, it is expressed and can directly give rise to a cancer. Researchers can insert DNA from a resulting cancer cell into a normal cell, and it too becomes cancerous. "These genes [missing in retinoblastoma victims] are different," Cavenee says. "They're normal genes, and you get

cancer by their absence. If you put these genes into a healthy cell, nothing would happen."

Since the 1983 meeting, researchers have linked the gene involved in the related cases of osteosarcoma to the same region of chromosome 13. And in studying the bone cancer they saw the same kind of genetic defect—loss of chromosome 13—in several youngsters with the disease. It's uncertain how these chromosomal events occur, but Cavenee thinks they may be tied to certain cell-growth processes.

In inherited retinoblastoma, a cell with a good and a bad chromosome appears to lose the good one during fetal or neonatal development, he says. At these stages, cells are undergoing repeated mitotic divisions and recombinations (exchanges of genetic material), and thus are particularly vulnerable to genetic loss. Likewise, the osteosarcomas are common during the teenage years, when there are large spurts of bone growth. Cavenee says the lost genes may play a role in regulating retinal and bone cell growth, and it's possible that without this proper control, the result is unbridled growth characteristic of cancer.

Geneticist Tom Shows of Roswell Park Memorial Institute in Buffalo, N.Y., also believes that such genes perform growth-related activities. They are present in every individual, he says, and cancer occurs when one of them malfunctions. "Growth-control genes are literally all over the genome [set of chromosomes]," says Shows, "and that's why there are so many kinds of cancer."

Meanwhile, researchers at Children's Hospital in Philadelphia have been looking at retinoblastoma children retrospectively in regard to second-site cancers. Anna Meadows, a pediatric oncologist who heads the study, says that contrary to earlier reports that listed the incidence of later cancers at nearly 40 percent, recent epidemiological data indicate that the figure is closer to 15 percent.

Meadows notes that the previous use of radiation — usually the treatment of choice over removal of one or both eyes—may be a factor in some of these teenage cancers. "Those kids who received radiation had a shorter period between diagnosis [of retinoblastoma] and their second tumor," she says. "It looks as if the radiation enhanced that and made it happen more rapidly."

Until about 15 years ago, doctors treated patients with low-energy radiation, which entailed a great deal of radiation scatter to other tissues — including bone, Meadows explains. Today, high-energy radiation, which can be applied more directly to the cancer site, is used. It's too early to tell if the switch in therapy will make a difference in the occurrence of subsequent tumors, she says, since most of those don't show up until 10 to 15 years down the road.

A gene elimination-duplication process similar to that of retinoblastoma has been implicated in the development of Wilms' tumor, an inherited kidney cancer that afflicts children, says Cavenee, who announced this finding at last September's March of Dimes meeting. Cavenee, who collaborated with investigators at Harvard and Johns Hopkins University in Baltimore, says the gene believed responsible for Wilms' is on chromosome 11, and as in retinoblastoma, it carries a deletion and has been identified in other cancers, notably in the liver. These findings of the past two years, he notes, have touched off an almost frantic search by scientists for cancers that work in similar fashions.

"I think a lot of cancers will be found to be caused by a similar type of gene," says ophthalmologist Ted Dryja of the Massachusetts Eye and Ear Infirmary in Boston. According to Dryja, only a handful of cancers are currently known to be hereditary. "One of the things that the retinoblastoma work has done is to give us a general method for looking at other cancers, like colon or breast," he says. "We'd like to see if the chromosomal loss mechanism applies there, too."

Says researcher Knudson: "Ultimately, we hope to find out how cancer works. We're getting close to that now, and in the near future I think we'll know how some do. It's a terribly exciting time." □

A prenatal test for cancer

Retinoblastoma, a heritable eye cancer, is the first such cancer to be linked to a damaged recessive gene that is expressed in the absence of its normal, dominant partner. In learning more about the way the disease works, researchers may have achieved another cancer first: a prenatal test to identify susceptible fetuses.

According to Webster Cavenee of the University of Cincinnati, if retinoblastoma strikes a parent and a child in a family, there's a very good chance that other brothers and sisters will also get the disease. To help find out, researchers at Childrens Hospital of Los Angeles and the University of California at Los Angeles early last year linked the gene for an enzyme, esterase D, to the retinoblastoma gene location. More recently, the University of Utah's Raymond White and Boston ophthalmologist Ted Dryja have pinpointed seven specific DNA fragments on chromosome 13 that they believe also lie close to the imprecisely located cancer gene. The key to using these DNA segments in prenatal cancer detection lies in whether or not they are indeed close enough to the retinoblastoma gene and, as a result, are inherited along with it.

Scientists can extract a lengthy piece of normal chromosome 13 DNA from an affected parent's heterozygous blood cell and chemically slice it into these tiny portions. Then they do the same to a strip of retinal cell tumor DNA from the parent and an affected son or daughter. Researchers can compare and ferret out the matching pieces, thereby determining which segments belong on the normal, undamaged chromosome 13, and which are present on the broken, disease-causing chromosome 13. These latter, shared strips of genetic material become the markers that scientists search for in the fetal cell DNA, obtained from amniotic fluid. This time, a match means the newborn will almost certainly develop retinoblastoma.

"It's the only case I know of where you can do prenatal diagnosis for the cancer developing before the fact," Cavenee remarks.

But he is quick to point out that the test is far from problem-free, and is still considered experimental. It has been tried in only a handful of cases so far. "Doctors are going to have to wait a few years to see if the predictions hold up," he says. "Otherwise there's no way of knowing if the test is any good."

Another question of the test's validity arises from the fact that Cavenee's group has observed the gene deletion defect in only a little more than 75 percent of the retinoblastoma tumors examined. "That suggests either we haven't been able to look closely enough at the other 25 percent, or there's another gene involved, on a completely different chromosome," he says. The latter explanation is unlikely, he believes. "If it turns out there are two genes, then the amniocentesis might not give the whole picture."

Offspring of former retinoblastoma patients are automatically tagged "at high risk," and Dryja says such children ought to be checked periodically for disease symptoms. Every three months until age 2 — and less frequently until age 5 — the child should be admitted to a hospital and examined under anesthesia. The procedure is expensive, and traumatic for the child, Dryja notes, and half the children don't get the disease. "The problem is we don't know which half," he says. Doctors hope an accurate prenatal test will eventually replace such exams, permitting earlier tumor detection and milder treatment.

There is as yet no comparable prenatal test for children of Wilms' tumor sufferers. Dramatic climbs in survival of the disease have come only within the last few years, Dryja explains, and "there haven't really been enough children available yet to study." Researchers hope to develop such a test within the next decade. —*S. I. Benowitz*