Sperm’s Chromosomal Contribution to Embryo and Infant Health

Also in this issue:
The 1995 R&D 100 Award Winners
The Genetic Contribution of Sperm: Healthy Baby or Not?

We are developing new methods to detect chromosomal mutations in sperm and early embryos. Our goal is to understand how defective chromosomes are induced in sperm and, when transmitted to an embryo from the father’s sperm, how they increase the risks of birth defects and childhood diseases, including cancer.
Atmospheric Hazards Project in the Environmental Programs Directorate were asked to determine what earthquake faults could affect the channel region and what earthquake forces might be expected in the area. While detailed seismic hazard assessments of the eastern portion of California’s Santa Barbara Channel, a region of high seismicity containing 17 oil platforms. Lab assesses offshore seismic hazards

The Laboratory in the News

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Shock compression used to study Jupiter

What astronomers believe to be the boundary between Jupiter’s molecular mantle and its metallic core may not exist. This is one of the possibilities outlined by Livermore scientist Bruce Nellis and two Livermore colleagues, Neil Holmes and Marvin Ross, in an article entitled “Temperature Measurements of Shock-Compressed Liquid Hydrogen: Implications for the Interior of Jupiter,” published September 1 in Science magazine.

"The only way on Earth you can achieve both the high temperatures and high pressures comparable to those inside Jupiter is by shock compression," Nellis said. For the research, Nellis and his colleagues used the Laboratory’s two-stage light-gas gun. High pressures and temperatures were generated by slaming tantalum or aluminum projectiles (accelerated to 7 kilometers per second, or 16,000 miles per hour) into sample holders containing liquid deuterium.

According to the team’s findings, the molecular envelope of Jupiter (which is 90% hydrogen) is cooler and has much less temperature variation than previously believed. Their data suggest there is no sharp change at what astronomers now commonly believe is a distinct core-mantle boundary. The analog on Earth is the distinct boundary between the rocky mantle and the iron core.

Nellis sees the publication of the Science article as the beginning of a new line of inquiry in the ongoing debate over the nature of the Jovian interior. The findings also will yield. These new results indicate that the equation of state of hydrogen is such that higher fusion yields are expected.

"Shock compression used to study Jupiter"

September 25 that President Clinton had decided to maintain nuclear weapons research at Lawrence Livermore, Los Alamos, and Sandia National Laboratories to preserve confidence in the nation’s nuclear weapons stockpile and ensure a sound technical foundation for seeking a Comprehensive Test Ban Treaty (CTBT).

In a signed directive, the President stated: “To meet the challenge of ensuring confidence in the safety and reliability of our stockpile, I have concluded that the continued vitality of all three DOE nuclear weapons laboratories will be essential.” The President said his decision in August to seek a "zero" yield CTBT “was based on assurances by the Secretary of Energy and the Directors of the Department of Energy’s nuclear weapons labs that we can meet the challenge of maintaining our nuclear deterrent under a CTBT through a Science-Based Stewardship Program without nuclear testing.” President Clinton added that the laboratories “can help change the course of history with respect to nuclear weapons.”

The Presidential decision, announced by Energy Secretary Hazel O'Leary in a Washington, D.C., press conference, was the first result of a major review begun May 4 of the federal labs belonging to the Departments of Defense and Energy and the National Aeronautics and Space Administration.

"I am extremely pleased with the President's confidence in the capability of the three Laboratories to provide for the national security through the stockpile stewardship program. The Laboratories and the Department of Energy have worked very hard to develop the program that made this decision possible," Bruce Tarter, Director Lawrence Livermore National Laboratory

Southon, a member of the Laboratory’s Center for Mass Spectrometry, said his principal role in the investigation has been to provide the expertise of LLNL’s radiocarbon group to confirm the conclusions reached by the primary USGS researchers.

"I am extremely pleased with the President’s confidence in the capability of the three Laboratories to provide for the national security through the stockpile stewardship program. Our conclusion was that the savings from consolidation would be insignificant compared with the impact that such action could have over time on our ability to maintain confidence in the nation’s nuclear weapons stockpile.” She added that Bill Perry, Secretary of Defense, strongly opposed the idea of transferring DOE nuclear weapons work to the Department of Defense, as some had proposed.

She said the Administration’s vote of confidence in LLNL was also influenced by the breadth of work conducted at Livermore, citing “firm cornerstones” in lasers, DOE’s Human Genome Project, and environmental technology. O’Leary pointed out that Brad Allenby, ATK’s research vice president for technology and environment, recently joined Livermore, “indicating that the Laboratory itself understands there’s a great future there.”

She said that the President "provided a powerful validation of the importance of the Department of Energy National Laboratories to the future security, prosperity, and well-being of our Nation.” DOE’s reform efforts, she said, are aimed at making the laboratories “more efficient and effective, while preserving their capacity for excellence in science and technology.”

"I am extremely pleased with the President’s confidence in the capability of the three Laboratories to provide for the national security through the stockpile stewardship program. The Laboratories and the Department of Energy have worked very hard to develop the program that made this decision possible," Bruce Tarter, Director Lawrence Livermore National Laboratory

The Laboratory is conducting an earthquake hazard assessment of the eastern portion of California’s Santa Barbara Channel, a region of high seismicity containing 17 oil platforms.

The work was requested by the California State Lands Commission and the U.S. Minerals Management Service as part of a project to reevaluate seismic response of offshore platforms. There are 31 oil platforms off the coast of California. Most date back to the 1960s and 1970s—before earthquake design methods and standards were established for the offshore oil industry.

Scientists and engineers from the Laboratory’s Geologic and Atmospheric Hazards Project in the Environmental Programs Directorate were asked to determine what earthquake faults could affect the channel region and what earthquake forces might be expected in the area. While detailed seismic hazard analyses already exist for onshore areas, there are currently no comparable analyses for offshore regions, such as the Santa Barbara Channel.

"Shock compression used to study Jupiter"

By the end of this year, Lab scientists should finish gathering information on faults in the Santa Barbara Channel area and on the seismic forces those faults could unleash. Next year, researchers will refine the data and develop a seismic hazards map of the region.

"Shock compression used to study Jupiter"

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Science & Technology Review November/December 1995
### Patents

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<td>Martin VanderLaan Larry R. Stankiew Bruce E. Walline Peter Petrovic Siegbert Gottisch</td>
<td>Method for Inimmunoassay Detection of Dioxins at Low Concentrations</td>
<td>U.S. Patent 5,429,925; july 4, 1995</td>
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<tr>
<td>Bernard T. Merritt</td>
<td>Modular Series Resonant Inverter</td>
<td>An electrochemical cell with a tapered cavity containing a quasi-stationary, permeable bed of electrochemically reactive particles. The dimensions of the cell cavity provide the bridging of particles across the cavity and the formation of voids to maintain a highly permeable bed. The particle serves 100% consumption of the particles.</td>
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<td>William A. Brummond Ravindra S. Upadhye Cesar O. Pruneda</td>
<td>Molten Salt Destruction of Explosive Waste Materials</td>
<td>A method for destroying energetic materials, including high explosives, propellants, and rocket fuels, by the formation of a reactive mixture, such as a sodium/potassium melt, which can be melted and recycled.</td>
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<td>Gary R. Dreyfuss Bernard T. Merritt</td>
<td>High Voltage Power Supply with Modular Series Resonant Inverter</td>
<td>A high-voltage power supply incorporating a plurality of phase-controlled, series-resonant half bridge inverters (modules) for minimizing harmonic distortion and for maximizing energy transmission.</td>
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<td>Seung J. Chang</td>
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<td>A self-filtering and self-imaging laser resonator that converts a low-divergence laser signal injected into the resonator into the desired resonator modes before the laser pulse starts. The laser cavity improves the quality of the resonator by the injection of the laser signal. The self-imaging property of the resonator reduces cavity-induced diffraction effects to improve laser beam quality.</td>
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<td>Sol P. Dijak Frank G. Patterson Robert J. Deri</td>
<td>Cross-Talk-Free, Low-Noise Optical Amplifier</td>
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### Commentary on Technological Vitality

The Laboratory is proud of its five R&D 100 award winners this year. The winners represent a cross section of our expertise in lasers, mass spectroscopy, electron beam processing, and aerogels and are an indicator of the technological vitality of our programs.

Our nation’s security needs drive the Laboratory’s missions, which in turn determine the technical questions and issues that our scientists must resolve. As they look for answers to difficult questions, they find themselves inventing new products, new processes, and even entirely new technologies. Our scientists do not set out to invent; rather, they look for the best way to deal with the problem at hand. Then, once a program has developed a new product that helps it carry out its mission, the product is often picked up by another program, modified, and put to a whole new use. This “spin-off” sometimes becomes a “spin-back,” returning to the original program where it is put to a new use altogether. Because of the Laboratory’s multiple missions, its programs generate more new ideas, products, and processes, and cross-fertilization frequently occurs.

The development of aerogels is an excellent example of the spin-offs and spin-backs that occur within the Laboratory. Aerogels are an ultra-light, solid material invented in the 1930s and studied at Stanford University. They were first put to practical use in the 1970s, when European physicists used them in nuclear particle detectors. Then, in 1980, a physicist at the Laboratory found that aerogels with some modification were useful in shock-wave studies to explore the physical properties of materials used in nuclear weapons. A few years later, inertial confinement fusion researchers at the Laboratory found that aerogels could absorb and hold liquid hydrogen (as a sugar cube absorbs coffee), which made them excellent fuel capsules in Shiva and Nova fusion targets. A few years later, aerogels spun back to the weapons program, where scientists found a new use that required the material scientists to expand their synthesis, characterization, and production capabilities for aerogel materials. A new level of precision machining was achieved for these lightweight, brittle materials, some of which were only a few times denser than air.

In the early 1990s, the Department of Energy determined that the know-how of the national laboratories should be made available to improve U.S. industrial competitiveness, and the Technology Transfer Initiative was born. Aerogels became one of the more widely applicable materials, with uses studied for insulation, filters, and batteries—NASA even studied them as a means of collecting micrometeoroids in space. This diversity of uses was largely a result of Laboratory efforts to develop and characterize new aerogel varieties and develop production facilities and machining techniques. About the same time, university researchers studying condensed matter discovered that these improved aerogels could be used to study the properties of superfluids, which are fluids that can flow with no resistance under ultra-cold conditions. Aerogels had spun back to their original use, as a tool for basic research.

Now, in the mid-1990s, aerogels have spun back yet again to the weapons program, where Laboratory scientists are using them in their science-based stockpile stewardship program. Used in improved shock-wave studies, aerogels help scientists explore the properties of materials under the conditions found in nuclear implosions and explosions. Most certainly, a number of our present and previous R&D 100 award winners can tell similar stories about the spin-offs and spin-backs of their inventions.

As products spin off from their original use, so have some Laboratory programs spun off from the core missions. The direction from the Atomic Energy Commission to the new laboratory in 1952 was to develop improved diagnostic techniques and study new types of weapons for our nuclear deterrent. Thus, early studies of the mutagenic effects of radiation on people handling test devices eventually led to our present biomedical research capabilities, which have made us a leading player today in the Human Genome Project. Likewise, early research to understand how radiation migrates through air, soil, and water led to the creation of our environmental programs, which are using this knowledge to develop site remediation technologies. These programs, while much more diverse and more broadly defined today than they were in 1952, still retain the common goal of serving our national needs.

The array of programs sponsored by the Laboratory is a key to our continued success, and R&D 100 awards are an acknowledgment of our continuing technical accomplishments.
Male animals—especially laboratory mice and rats—that are exposed to certain damaging agents can suffer adverse reproductive effects and other outcomes. For decades, we have known that certain occupations and environmental exposures are linked to abnormal reproductive outcomes. Some risks can date back to the time of conception of either parent. Livermore researchers are focusing on defects in sperm that lead to abnormal outcomes.

### Three Kinds of Evidence

#### Factors affecting development of sperm, egg, and embryo

- Diet
- Occupation
- Random errors
- Environment
- Inherited factors
- Toxic drugs
- Genetic susceptibilities

#### Abnormal reproductive outcomes

- Infertility
- Pregnancy loss
- Abdominal pregnancy
- Birth defect
- Childhood cancer

#### Normal, healthy baby

**Father’s sperm**

**Mother’s egg**

**Fertilization**

**Embryo development**

**Abnormal, unhealthy baby**

Figure 1. Many risk factors (top of diagram) acting on the mother, father, and offspring may lead to abnormal reproductive outcomes. (Bottom right). Some risks can date back to the time of conception of either parent. Livermore researchers are focusing on defects in sperm that lead to abnormal outcomes.

### Declining Sperm Count

First, the sperm count of men has been declining over the last five decades, and we still do not know exactly what accounts for the decline. In 1983, we conducted studies for the U.S. Environmental Protection Agency (EPA) on the effects of nearly one hundred different types of exposures on sperm production in human males. About half of the agents we studied, including alcoholic beverages, cigarette smoke, and lead, lowered the production of sperm or affected sperm motility or morphology.

#### Occupational Exposure

A second important line of evidence suggests that certain jobs and workplace and environmental exposures of the father are linked to spontaneous abortion and problems in their offspring.

### Genetic Mutations in Infants

A third line of evidence for male-mediated reproductive effects comes from studies of babies when some of the newest molecular methods are applied. Such studies show that while entirely genetic mechanisms are involved in an offspring—ones never seen before in either the mother’s or the father’s family—they are almost always associated with the father’s genes. Several defects also predominantly occur in the father’s chromosomes. For example, about 80% of the chromosomal aberrations seen in the chromosomes of babies—defects such as chromosome breaks—come from the father.

### Confusing Evidence

For decades, we have known that male animals—especially laboratory mice and rats—that are exposed to certain damaging agents can suffer adverse reproductive effects and other...
health problems. The effects can include reduced sperm production, diminished quality of sperm, and reduced libido.

Researchers can systematically study rodents to gain a better understanding of the links between exposure and reproductive effects. However, for humans, we must rely on the few sources of evidence that are available to us, including exposed individuals and their offspring. Studies since the 1950s have consistently shown that exposures of human males to environmental, occupational, or therapeutic agents can have detrimental effects on sperm count, motility, or shape. In contrast, although many environmental agents clearly have mutagenic potential in animals, experts have disagreed on whether environmental exposure of human males contributes very much to genetic disease or to adverse effects in their offspring.

For example, the research literature is consistent in confirming the adverse genetic effects of ionizing radiation in the male mouse and its offspring. However, the human offspring of atomic bomb survivors have no measurable increase in induced mutations. Similarly, exposure to various agents used in chemotherapy and to radiation therapy do not yield clear-cut results for genetic effects in the offspring of treated male patients.

Some of the puzzling inconsistencies between humans and mice may be due to individual variation and species differences. Other explanations involve the role of DNA repair processes or the possibility that some chemical or physical agents (mutagens) may have limited or short-term effects on sperm.

Human doses are often small compared to those used in research on mice, and the number of human offspring that have been studied for induced genetic effects remains relatively small. Finally, it is possible that the types of genetic damage (called “endpoints” by geneticists) assessed in studies of exposed humans are not sensitive enough to always reveal a significant effect.

A Review of the Basics

The body (or somatic) cells of humans and other mammals contain pairs of chromosomes. Except for the sperm or egg cells and red blood cells, human somatic cells carry 46 chromosomes (the diploid number). Normal human somatic cells have 22 pairs of autosomes (nonsex chromosomes) and one pair of sex chromosomes, either XX or XY. Of the sex chromosomes, a normal female carries two X chromosomes, and a normal male carries one X and one Y chromosome.

In contrast to somatic cells, each sperm and egg contains 23 chromosomes (the haploid number in humans). Each normal sperm and egg carries one copy of chromosome 1, one copy of chromosome 2, and so forth. Figure 2 shows the haploid number of chromosomes from human sperm that were specially prepared by a technique developed at the Laboratory.

If either of the germ cells carries an abnormal number of chromosomes or some other genetic defect, major hazards may arise for the offspring. A fetus resulting, ... method for detecting chromosome abnormalities in sperm has broad implications for maintaining or improving human health.

About Aneuploidy

The measure (or biological marker of male reproductive risk) we have chosen to study in depth is sperm aneuploidy. Aneuploidies in general are an important category of chromosomal damage that can be transmitted to the offspring from either the father or mother. The word “aneuploidy” refers to cells carrying the wrong (i.e., the prefix “an-”) number of chromosomes (“euploid”). Aneuploidy is one of the most common and serious chromosomal abnormalities recognized in humans. It is responsible for a large portion of infertility, pregnancy loss, infant death, malformations, mental retardation, and behavioral abnormalities.

Human embryos with an abnormal number of sex chromosomes or with an extra chromosome 13, 18, or 21 can survive to birth and beyond. An extra chromosome 21 causes Down syndrome and is a familiar example of aneuploidy involving one of the nonsex chromosomes. However, the most common aneuploidies in humans at birth involve an abnormal number of X or Y chromosomes. This condition, sex-chromosome aneuploidy, can be diagnosed prenatally through amniocentesis, and the incidence is about 1 in 250.

Table 1 shows different types of sex-chromosome aneuploidies together with other abnormalities involving the autosomes. A male child who inherits, say, an extra Y chromosome from the father would have a total of 47 chromosomes and a sex-chromosome aneuploidy (XXX). We know that human fathers are responsible for 100% of 47, XXX cases because the mother carries no Y chromosome. Another aneuploidy involving the sex chromosomes is Turner syndrome (45, XO), in which a paternal chromosome is lacking about 80% of the time. Other conditions are Klinefelter syndrome (47, XXX) and a triplet of X chromosomes (47, XXX).

Slightly more than half of the sex-chromosome aneuploidies at birth
are of paternal origin. The effects of such aneuploidy depend on which combination of X and Y chromosomes is involved. The health effects of XYY, for example, are minor; however, the effects of Turner and Klinefelter syndrome include physical, behavioral, and intellectual impairment as well as sterility.

Among human babies, the frequency of known chromosomal abnormalities, including aneuploidies and structural aberrations, is about 0.6%. In addition, about 1% of newborns carry a mutation for a genetic disease. When an inherited error occurs, we need some way to ascertain when the condition is due to the father, what factors can cause the condition, and what prevention strategies might be effective.

The DNA complex forming the chromosomes (chromatin) in sperm is typically rigid and so dense that it occupies nearly the minimum possible volume. The high degree of condensation makes it nearly impossible to visualize and identify individual chromosomes by standard microscopy. The two photos show how our hamster technique has become a highly reliable tool, and we consider it to be the “gold standard” against which we evaluate any new methods. However, the technique is difficult to apply and is both labor-intensive and inefficient, so it is costly to perform.

First Use of FISH in Sperm

In our first demonstration of FISH in human sperm, we applied a fluorescently labeled DNA probe to the Y chromosomes of sperm from human volunteers. As shown in Figure 4a, the Y chromosomes were tagged with a fluorescein label, a green-fluorescing dye that can look yellow, for example, on a red background. The Y chromosomes are easily recognized as bright yellow spots, called “domains.” We counterstained the sperm nuclei with the red-fluorescing dye propidium iodide, which produces the bright red background color.

After examining and scoring 11,500 sperm nuclei, we found that 50% of sperm showed fluorescent domains consistent with the presence of a Y chromosome. The proportion is what we would expect, because about half of all sperm carry a Y chromosome, and half carry an X chromosome. This finding is also consistent with the proportion of sperm containing Y chromosomes as determined by the hamster technique. As anticipated, FISH proved to be a direct and reproducible method for monitoring the chromosome constitution of sperm, and it allows us to visualize thousands of cells rapidly. In subsequent studies, we expanded the number of DNA probes we can apply to sperm nuclei, allowing us to tag two or three different chromosomes simultaneously, and we extended the method for use in laboratory animals.

Assessing Damage

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The Hamster Technique

In 1978, Rudak and colleagues working in Hawaii pioneered a way to analyze the chromosomes in human sperm after fusion with hamster eggs. Until recently, this “hamster technique” was the only method available for characterizing chromosomal defects in human sperm. During the 1980s, LLNL researchers were the first to get the hamster technique to reliably work for a variety of applications, a considerable challenge because biologists elsewhere in the world were having major difficulties in obtaining usable results. The March 1984 issue of *Energy and Technology Review* provides a more complete description of this highly useful tool. The hamster technique gives valuable baseline information on the normal burden of damage in healthy men. Using the method, we found that a small proportion of sperm in otherwise healthy males carries aneuploidies or other types of structural aberrations.

The hamster technique has become a highly reliable tool, and we consider it to be the “gold standard” against which we evaluate any new methods. However, the technique is difficult to apply and is both labor-intensive and inefficient, so it is costly to perform.

Fluorescence In Situ Hybridization

By about 1990, the Laboratory had developed a new biological procedure that could detect aneuploid sperm more efficiently than the hamster technique. Fluorescence in situ hybridization (FISH) has been previously described in *Energy and Technology Review* (see the April/May 1992 issue) as a gene-mapping tool. The method is illustrated in the context of our sperm research in the box on page 12. In essence, we prepare chemically labeled DNA probes and bind (hybridize) them to target chromosomal DNA within the sperm head. During the 1980s, LLNL researchers were the first to get

Three-Probe Assays

Next, we added a fluorescently labeled DNA probe for one of the autosomes in sperm. Whereas any autosome would suit the purpose, we selected a probe for chromosome 8, which was our best DNA probe available at the time. Adding one autosome to FISH is a major advantage because it allows us to distinguish among three possibilities: duplication of a sex chromosome only (sex-chromosome aneuploidy), duplication of a single autosome only (autosomal aneuploidy), and duplication of the

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entire genome (diploidy). In other words, if we detected the condition XX88 or YY88, then it is highly likely that all the other chromosomes are duplicated as well (the diploid condition).

Figure 5 illustrates our three-probe assay for chromosomes 8 (yellow), X (green), and Y (red). Figure 5a shows normal human sperm, which carry either X8 or Y8. All such normal sperm fluoresce in only two colors and show two domains (two discrete fluorescent areas). Abnormal human sperm in Figures 5b through 5j show more than two domains (for example, XX88 has four domains) or more than two colors (for example, XY8 has three colors and three domains).

As more FISH probes become available, we will add them to the assay. As soon as an excellent probe for chromosome 21 is developed, we will include it in the FISH assay so that we can look for this important marker for sperm that may lead to Down’s syndrome. Similarly, we will soon add DNA probes for chromosomes 13 and 18 because these trisomies, like chromosome 21, survive to birth and beyond in humans.

Recently, we developed another useful tool that has important implications. By adding the technique of phase-contrast imaging to fluorescence microscopy, we can now differentiate sperm carrying XY88, for example. Such an arrangement could represent either a diploid sperm or a normal somatic cell (which always carries two sex chromosomes and copies of each autosome). Phase contrast allows us to differentiate clearly between the two possibilities because somatic cells have no tail.

Validating the Method

To demonstrate the utility of the FISH method for assessing sperm chromosomes in humans, we needed to address the issue of validation. How would we know the results of the FISH assay are actually correct? Fortunately, several lines of evidence from independent sources can be used to validate our assay.

Researchers at Livermore Laboratory and in Canada and Japan have used the hamster technique to collect baseline information establishing the normal ranges of sperm chromosome numbers. They have also used this technique to validate the FISH method. In addition, we have used the FISH method to assess sperm chromosomes in humans and have found that the results are consistent with those obtained using the hamster technique.

Figure 5. Our three-probe FISH procedure applies a mixture of probes specific for chromosomes X, Y, and 8, each tagged with a different fluorescent dye. Here, human chromosomes X fluoresce green, Y fluoresce red, and 8 fluoresce yellow. (a) Normal sperm carry either X8 or Y8 and are marked by only two different colors and two domains. (b-f) Abnormal sperm, such as XX8, YY8, XY8, X88, or Y88, have three domains but of varying colors. (g-j) Abnormal sperm, such as XXBB or YYBB, have two colors but four domains. (h) and (i) show the sperm tail using phase-contrast imaging.
burden of chromosome damage in human sperm. These studies suggest that the baseline frequency of aneuploid sperm in young, healthy males is 3 per 10,000 chromosomes. This is the reference value we used in assessing the new FISH assay. We also have hamster data on the frequency of abnormal chromosomes after administering doses of some mutagenic drugs. Finally, we have assessed chromosomes X, Y, 1, and 8 for evidence of aneuploidy in hundreds of thousands of cells from healthy men. The frequencies of aneuploid sperm can vary among the different chromosome types and among individual male donors. Furthermore, most healthy men give consistent results over time (up to four years).

Our assays show that human sperm contain the abnormal chromosome pairs 8-8, XX, YY, and XY at frequencies of roughly one per 2,000 sperm analyzed, averaging across donors. This frequency is quite similar to the value of 0.6 per 2,000 sperm obtained by the hamster technique. We found that the abnormal chromosome pairs 1-1 had the highest frequency of all, about 3 per 2,000 sperm. No sperm of the many thousands we tested from different healthy donors contains more than two of the same chromosome type (for example, we do not find the triplets 888, 111, or YYY). The most common sex chromosomal abnormality we found using the three-probe assay was XY8, with an average frequency of 9.5 per 10,000 human sperm scored.8 Table 2 summarizes the frequencies of abnormal sperm types we found in studies of young, healthy, human males using FISH.

### Bridging Biomarkers

We recently developed corollary methods for detecting aneuploidy in the sperm of mice and rats using two- and three-probe FISH. We used a multicolor FISH procedure to evaluate chromosomes X and 8 in more than 80,000 sperm from healthy, young adult mice. About 3 sperm per 10,000 cells evaluated showed XX or 88 aneuploidies (Figure 7). The frequencies we found for these particular numerical errors in two strains of mice were indistinguishable from those for sperm from healthy men using similar procedures and scoring methods. This work serves to demonstrate what we call “bridging biomarkers” between humans and animals for detecting sperm aneuploidy.

Bridging biomarkers, in essence, allow us to use the same type of measurable variation as we assess similarities and differences among species. With this type of information, we can compare the mean error rate of specific chromosomal defects in human versus rodent sperm, especially the sperm of mice. Our data show that the mean error rate is about the same in otherwise healthy male mice and humans. The bridging biomarkers of sperm aneuploidy also allow us to compare human and laboratory species for effects of physiological changes (e.g., diet, age), effects of exposure to toxins, and effects of genetic differences. The studies of age effects are summarized to illustrate the utility of bridging biomarkers.

### Effects of Smoking

Cigarette smoking is one of the most pervasive examples of the self-administration of toxic compounds. Research over several decades at Livermore and elsewhere has shown that cigarette smoking can cause defects in sperm quality. However, no information has been available on its mutagenic potential in sperm.

Our findings on age effects in men are strikingly similar to our recent results on aneuploid sperm in aged mice. Aged mice (mice normally live for about two years) had about twice as many aneuploidies of the types XX8, YY8, 88X, and 88Y than did younger mice (slightly older than two months). As with human males, we found the largest age-related increases in the XX8 and YY8 aneuploidies (Figure 8). If our findings on aging continue to hold up with further research, they may point to an intriguing possibility. According to several lines of evidence on the production of sperm and egg cells with genetic errors, age effects in human females are predominant in the first stage of meiosis (the first of a two-stage process in forming an egg or sperm). Our preliminary data on both mice and human males suggest that age effects in males are predominant at the second stage of meiosis. Thus, males and females may differ in terms of the exact stage at which some genetic errors, such as aneuploidy, arise.
We recently studied 15 smokers and 15 nonsmokers from the Czech Republic and found that smokers produce approximately twice the number of aneuploid sperm as nonsmokers. Cigarette smoking is a life-style that often includes alcohol consumption and possible stress factors. Thus, further research is needed to determine whether the effects we found are indeed due to tobacco products or to other aspects of a smoker’s life-style.

Effects of Chemotherapy

We have applied the three-probe FISH method to sperm cells of cancer patients before, during, and after treatment with the combination chemotherapy NOVP. Our basic question was whether the aneuploidies induced in sperm might persist following treatment, raising the possibility that genetic damage could be passed on to future offspring. We elected to study NOVP treatment because it contains drugs known to produce aneuploidy in model systems.

Figure 9 shows our results for young male patients with Hodgkin’s disease (a kind of lymphatic cancer). When compared with healthy controls, these patients had elevated frequencies of aneuploid chromosomes X, Y, and 8 even before treatment (twofold to sixfold increases over normal levels). Just after NOVP treatment, the frequencies of numerical abnormalities increased twofold to fivefold compared to pretreatment levels. Following chemotherapy, aneuploid sperm returned to pretreatment levels within two to six months—clear evidence that at least one type of chemotherapy has transient effects on aneuploidy in human sperm. Further studies are needed to determine whether other drugs induce aneuploidy in human sperm and whether the effects are also transient.

New Research on Embryos

In very recent work, we have begun to assess damage in embryos, using the mouse as the model species. Figure 10 summarizes the various developmental stages of the mouse embryo at which we can now apply the FISH method to assess numerical and other errors in chromosomes. For example, we can study the embryos immediately after fertilization (just before first cleavage of the embryo when it divides into two cells), at the two-cell stage, or about four days later just before the embryo implants into the uterine wall and when it consists of 30 to 50 cells. This work has required the development of special FISH probes and techniques that are suitable for embryos.

One of the advantages of studying one-cell mouse embryos (called “zygotes”) is that the chromosomes from the paternal and maternal mice...
Our new studies on four-day mouse embryos (Figures 11d and e) are a collaboration with the University of California at Berkeley. Very little work has been done on such embryos, so our research will be among the first. We are applying FISH and other biological imaging methods to understand how the development and survival of implanted embryos are affected by mutation exposure of the father’s sperm before mating. Acrylamide is the model mutagen for our four-day embryo project because it is known to induce heritable defects. The damage we are seeing in embryos includes aneuploidy, mosaics (a combination of some normal cells and some chromosomally altered cells), chromosome breakage, and polyploidy (the occurrence of chromosomes that are three or more times the haploid number). We expect that the methods will also be useful for future studies on the outcomes of human in vitro fertilization.

New Research on Klinefelter Syndrome

We are beginning to look at blood samples from humans who carry the genetic abnormality associated with Klinefelter syndrome (47, XXY). Such individuals tend to be shorter than normal in physical and behavioral development, they eventually grow taller on average, and they are all sterile. The work is a collaboration involving LLNL and five other institutions.

About half of 47, XXY cases receive the extra X chromosome from the father (such aneuploid sperm would carry both the X and Y chromosomes). Male children with this syndrome and their fathers provide us with a unique opportunity to learn about the relation between sperm aneuploidy and aneuploidy at birth. We will study 40 families with children whose diagnosis of Klinefelter syndrome has been genetically confirmed. We want to know if fathers who are responsible for the syndrome in their child produce inherently elevated levels of aneuploid sperm, especially X-XY sperm. To increase the speed of scoring defective chromosomes and the use of objective criteria in the FISH assay, we are also developing new automation and image-analysis techniques.

Looking Ahead

For decades, genetics researchers, concerned physicians, and many parents have struggled to come to terms with the causes and conditions that may underlie abnormal reproductive outcomes. With highly efficient FISH probes, we are beginning to understand some of the ways chromosomal abnormalities can arise in sperm and how those defects may lead to defects in the embryo.

Our expectation is that the new procedures will lead to a far greater understanding of the relations among certain genetic defects in human sperm, the effects of age, environmental exposures, and life-style factors, and the probability of fathering a chromosomally defective child. On the horizon are improved FISH assays for more chromosomes, new assays that can detect chromosome breakage in sperm, and automation and objective image processing. Such advances will help to make our methods more accessible to the rest of the research community.

Key Words: aneuploidy, chromosomal abnormality; DNA probes; fluorescence in situ hybridization (FISH); Klinefelter syndrome; sperm—human, rodent, sex chromosomes.

References


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About the Scientist

ANDY WYROBEK joined the Biomedical Sciences Division of the Laboratory in 1975. He is currently the principal investigator of the sperm and embryo research team within the Biology and Biotechnology Research Program at the Laboratory. He received his B.S. in physics from the University of Notre Dame in 1970 and his Ph.D. in medical biophysics from the Ontario Cancer Institute at the University of Toronto in 1975. In more than 80 publications, Andy Wyrobek has explored male-mediated developmental toxicology, human male reproductive hazards, and mammalian testing systems for detecting the genetic effects of environmental, occupational, and therapeutic agents in sperm and embryos. His special interests are understanding the mechanisms leading to birth defects and identifying the environmental and genetic risk factors for abnormal pregnancies.
R&D 100 Awards Recognize Five Laboratory Inventions

The Laboratory has done it again. In receiving five R&D 100 awards, the “Oscars” of applied research, the Laboratory has demonstrated its stature among the premier research institutions in the world.

Every year, scientists from corporations, government laboratories, private research institutes, and universities all over the world submit entry materials to R&D Magazine, vying for an award. Editors of the magazine and a panel of experts judge the entries, looking for the most technologically significant products and processes, ones that promise to change people’s lives for the better.

Many past winners have become part of our everyday lives—Polacolor film, the halogen lamp, anti-lock brakes, the automated teller machine, the fax machine, the nicotine patch, and color computer printers. The Laboratory has won 53 R&D 100 awards since the competition began in 1963, including the过程 for the diamond turning of optics (1978), the three-dimensional chemical x-ray microscope (1988), and the hard x-ray lens (1991).

There is no telling what can happen to an R&D 100 award winner. In 1993, the Laboratory won an award for the world’s fastest solid-state digitizer, a product that grew out of research related to the Nova laser. Today that digitizer has been put to use in micropropulsion imaging radar (MIR), a $10 to $15 million system that can do a job that used to require equipment costing up to $40,000. MIR can be used on automobiles to warn of collisions, can detect buried land mines, and can help rescuers searching for people trapped alive in destroyed or collapsed buildings. The range of MIR applications is so broad that the Laboratory is selling licenses to apply MIR technology in 14 different areas.

This year’s awards were given to six teams of Laboratory scientists who made major progress in the fields of aerogels, lasers, mass spectrometry, and electron beam processing.

The final two awards were given for developments in fields as diverse as mass spectrometry and electron beam processing:

- The first hand-portable mass spectrometer, based on the principle of ion cyclotron motion, combines the ion source and mass analyzer/detector with an integral vacuum system. In spite of its small size and simplicity, the system can achieve sufficient resolution to detect trace compounds or contaminants in air samples.
- A small, inexpensive electron beam gun can be used to process paints, inks, computer floppy disks, medical supplies, and other materials. The beam exits the gun through a new, thin-film window and delivers higher beam energies much more efficiently than large, expensive, conventional electron beam systems.

The group of articles that follows highlights each of these R&D 100 award-winning inventions and introduces you to the men and women of Lawrence Livermore National Laboratory who made them possible.
Aerogels are among the lightest solids known, with some varieties consisting of 99.8% air. Many aerogels are nearly transparent and are called “frozen smoke” for their ghostly appearance. At the same time, of all known solids, aerogels have the highest internal surface areas per gram of material, thanks to their complicated, cross-linked internal molecular structure. An aerogel the size of a grape has the surface area of about two basketball courts. The internal microstructures give aerogels exceptional strength—some aerogels can support 1600 times their own weight.

First made in 1931, silica aerogel, composed mainly of silicon dioxide (sand), is probably the best known type of aerogel. Another type is organic aerogel, made up mainly of carbon and hydrogen atoms. At Lawrence Livermore National Laboratory, one of the world’s leading centers of both silica and organic aerogel research and development, scientists attracted international attention early in this decade when they created a silica aerogel some 10 times less dense than the previous lightest version.

While the mold is rapidly heated, the liquids react to form the gel. The gel’s shape is defined by the mold walls, which keep it from straining under the influence of the rising hydrostatic pressure of the liquid within the gel. After as little as 20 minutes, the temperature and pressure within the mold produce the supercritical fluids, which are rapidly purged from the confined gel without cracking it. The internal pressure in the mold is rapidly lowered by releasing it through an opening. After the mold is cooled for a few minutes, it is opened and the finished aerogel part is removed. The entire process, from start to finish, takes minutes instead of the hours (or even days) required by conventional processes, and the finished aerogels require no further machining or polishing.

“Our process is the only one that can mass-produce aerogels of precise sizes and shapes while maintaining high surface tolerances,” says Hrubesh. “This is possible because the mold totally defines the size, shape, and surface quality of the aerogel object. Other processes require at least one free surface, which can distort during supercritical drying.”

The Livermore team estimates that the cost for aerogels made using the new process is less than $3 per liter, as opposed to the $25-per-liter price of aerogels available today using conventional processes. Furthermore, the new process reduces liquid waste by 40% over other methods because the liquid purged from the gel can be reused to make more aerogels. Finally, the process uses about 10 times less energy than other techniques because it does not require the pumping of fluids and because it heats the molds for only a short time.

The Livermore aerogel processing breakthrough was aided by the analytical work of Paul Coronado and John Poco, who fabricated double-wall containers with thermocouples inside to monitor the pressure and temperature of the gelling process. Using these containers led to a much greater understanding of aerogel chemistry and the discovery that changing small amounts of starting ingredients has a significant effect on the gelation and drying processes.

The research team is continuing to refine the injection molding process. Taking advantage of the new technology, team members are also making small double-paned windows containing aerogels, one of which has a thermal insulating value of R-19, equivalent to the insulation value of a house wall backed with a 8.75-cm-thick roll of fiberglass.

Aerogels for Purifying

One of the most promising new applications for aerogels is in a cost-effective water purification process developed at the Livermore laboratory. After removing larger solids and particle by gravity settling, the water is further purged of smaller impurities by passing it through a bed of aerogel material. The filter lasts at least 20 times longer than conventional filters, and the resulting water is clear and potable.

A Shared Award in Aerogel Process Technology

Getting Aerogels into Shape

A major stumbling block to the commercialization of aerogel products is the slow and labor-intensive manufacturing process. In response, a team of Laboratory researchers developed a process for producing shaped aerogel parts that is more than 30 times faster than conventional methods.

The injection molding process for net-shaped aerogels uses various molds (samples shown) to rapidly produce silica aerogels in a variety of shapes.
Farmer says that more research and development is needed before carbon aerogel CDI technology can be incorporated into any large-scale plant. Future efforts will include component aging tests, more precise energy analyses, and experiments with high concentrations of various salts as well as acidic and basic electrolytes. Finally, cost-effective, high-volume production processes are needed for carbon aerogel electrodes to achieve their practical benefits.

The Livermore research team experimented with various salt concentrations and operating voltages and showed that the system is capable of removing 95% of salt before the carbon aerogel electrodes become saturated. After several months of operation at 1.2 volts per cell (judged the most effective setting), the electrodes lost only 6 to 8% of their capacity.

Perhaps the most important application involves treating liquids containing radioisotopes. Farmer notes that both the U.S. Department of Energy and the former Soviet Union have large inventories of solutions contaminated with radioactive materials. Unlike ion exchange, carbon aerogel CDI can treat these radioactive wastes without generating secondary wastes.

Farmer says carbon aerogel CDI may offer significant advantages over competing methods (such as reverse osmosis and ion exchange), which typically consume large amounts of energy, involve costly and often troublesome membranes or high-pressure pumps, and often generate large quantities of corrosive wastes that must then be specially treated. For example, ion exchange columns, commonly used to remove heavy metals and radioisotopes from waste water, require about 100 kg of acid to regenerate 1 kg of cation exchange resin. In contrast, carbon aerogel CDI regenerates its cells by electrically discharging them; the cells are then rinsed with a small volume of water.

One of the most interesting potential applications for carbon aerogel CDI is desalinization of brackish water (containing typically 800 to 3200 ppm salt) for residential, commercial, and agricultural purposes. Preliminary studies show that the process may require less energy than other competing technologies because it does not employ complex membranes and does not require flow-through porous media. At some point in the future, it also may be possible to treat sea water (35,000 ppm salt), an important application for many parched California coast communities.

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LASER beams do not just happen. Atoms must be pumped with light energy to “excite” them. As excitation continues, the energy in the atoms increases, and the atoms give off energy in the form of light. The light can be visible light or invisible light like infrared or ultraviolet light. Depending on the type of laser, the excited atoms may be in a gas or a solid material, and the pumping mechanism may take a variety of forms.

Flashlamps have been used for excitation since the birth of lasers in the early 1960s. But flashlamps need to be replaced frequently, and they do not deliver energy very efficiently. So, about 15 years ago, scientists began to investigate the diode, a semiconductor device that converts electric power directly into light, as a pumping device for some solid-state lasers. The diode pump source began to be widely used about five years ago. Yet, diode-pumped lasers had drawbacks, too. While the diodes could produce laser light in high-intensity pulses, their overall power output was low (less than 1 kW/cm²), which reduced their usefulness.

A team of Laboratory scientists led by Ray Beach hit upon a remarkably simple solution to the low power output problem. “We imagined a ‘light funnel,’” says Beach, “wherein the output from the diodes is captured and manipulated to concentrate the pump light.” This light funnel is a small lens for concentrating, or “conditioning,” the output radiation of the diodes. With higher power outputs, other advances became possible. Previously, applications requiring high-average power were limited to a 1-micrometer wavelength beam when using a diode-pumped, solid-state laser. But with this new lens technology, a broader range of solid materials can be used that produce a variety of wavelengths, increasing the applications of these lasers. A 2-µm beam is even more useful. In surgery, for instance, a 2-µm beam causes collateral damage to tissue around surgical area. A 2-µm beam, on the other hand, cuts and cauterizes the surgical area while causing much less collateral damage to surrounding tissue. A 2-µm beam also has a variety of remote sensing applications—it can be mounted on an airplane to detect wind shear or placed in a suspected area to detect the byproducts of chemical or biological warfare.

With a light funnel lens, the output power of diode-pumped, solid-state lasers is increased by a factor of 250, and their unit cost is reduced by a factor of 100. “Our product offers a simple, inexpensive, and commercially attractive technique for scaling up the output power of laser systems,” says Beach. “And this ability to scale up the laser’s output power drastically reduces its price.”

The Lens Conditions the Light

The three components of the Laboratory team’s all-solid-state system are a laser diode pump array, the light funnel or lens duct, and a laser crystal. The lens duct (shown above) lies at the heart of this new design. It condenses and homogenizes the diverging light output from the diode array, coupling the diode light to the laser material at the lens duct’s exit face. The pie-shaped lens duct can be fabricated of inexpensive glass or plastic. Its longer input face and shorter output face are coated with an antireflective material for more efficient transmission of light. The other four sides are uncoated and prevent light from escaping by reflective wave guiding, which is produced by internal reflection of the light beam. While this optical device is particularly applicable to coupling diode array pump light to solid-state laser materials, it can be applied to any situation that requires focusing a beam of light to produce a smaller beam of greater intensity. This lens duct can be used to scale up both pulsed and average-power laser systems. The team has developed a pulsed, 1-µm, diode-pumped, solid-state laser with an output pulse energy of 100,000 microjoules (µJ). The nearest competitor produces just 400 µJ of output energy. This 250-fold gain in output pulse energy also costs much less to produce: $1 per microjoule compared to that competitor’s $100 per microjoule. Other systems on the market produce even less output pulse energy at a higher cost per microjoule.

An average-power, 1-µm laser also performs markedly better than those of the competition. The Laboratory’s system produces 13 W of output power, which is two and a half times what the nearest competitor can produce and at a much lower cost.

A 2-µm unit developed by the Laboratory team produces 25 W of average output power. A diode-pumped, solid-state laser producing this much power at the 2-µm wavelength was not possible before the development of the lens, and as a consequence, no comparable commercial system exists.

Applications from Medicine to Manufacturing

Applications for this new class of lasers abound in medicine, radar, manufacturing, and materials processing. The average-power, 2-µm system was developed as a surgical laser. The $78-million-per-year medical laser market is presently dominated by flashlamp-pumped lasers, which could not produce 25 W of average output power. A diode-pumped, solid-state laser producing this much power at the 2-µm wavelength was not possible before the development of the lens, and as a consequence, no comparable commercial system exists.

Looking even further into the future, megawatt-class lasers could be used for laser-driven, inertial confinement fusion power plants. In fact, Laboratory scientists developed one of their 1-µm systems as a subscale prototype for this application. Until very recently, scientists thought that solid-state lasers, even under ideal conditions, did not provide adequate efficiency and robustness for a commercial power plant setting. But by extending the current laser fusion technology base to include this lens duct technology and other advancements in solid-state lasers, a laser fusion driver concept appears feasible for the first time.

The diode-pumped, solid-state laser was developed under Cooperative Research and Development Agreements (CRADAs) with the Beckman Laser Institute and Medical Clinic at the University of California at Irvine, and Wellman Laboratories of Photomedicine, Boston, Massachusetts.
High-average-power solid-state laser developers (from left) Brent Dane, Lloyd Hackel, and Mary Norton with an advanced, two-amplifier prototype of their invention in the laboratory. Using recently improved amplifier glass developed with Schott Technologies, this laser should exceed the average power of the award-winning system by a factor of 4.

A Unique Amplifier

A new type of laser amplifier consisting of mirrors, rotators, polarizers, special laser glass, and other optical devices is behind all of these “firsts.” All materials used in the amplifier have high damage thresholds to withstand the extremely high energies. A version of the laser, operating at around 100 joules per pulse, could provide the pump source for a tabletop-size x-ray laser, whose coherent output would be used to produce very-high-resolution, three-dimensional images.

The team has also discovered that high-energy, short-pulse lasers are excellent for quickly removing paint from surfaces with an intense acoustic wave that does not harm the surface beneath the paint. This is an environmentally sound method of removing paint from aircraft and ships, lead-based paint from public and private buildings, and painted graffiti from a variety of surfaces. “Graffiti removal is a hot topic,” notes Dane, “and is being explored further by other scientists at the Laboratory.”

To obtain even higher average power output from the laser, a new laser glass with much higher mechanical strength has been developed that could double the average power of a single amplifier. Furthermore, the use of SBS phase conjugation allows multiple, sequentially firing amplifier heads to be combined in a single laser beam train. A two-head laboratory prototype is currently in operation, and a version of the laser using four amplifier heads is under construction. The average power of the latter is expected to easily exceed 1,000 W.

Lighting Launches and Removing Graffiti

High-resolution speckle imaging and x-ray lithography are just two applications for this new laser. These frequency-doubled green light outputs of this system, with its narrow bandwidth and accurate long-range pointing stability, is ideal for advanced laser radar uses. For the U.S. Navy, the team developed a high-energy, green-output laser with a 500-nsec pulse duration and delivered it to the Navy at Cape Canaveral, where it will be used to illuminate rocket launches from the Kennedy Space Center. During independent testing prior to shipment, the laser fired over one million shots with no maintenance or realignment required.

One of the World’s Brightest Lasers

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For further information contact Brent Dane (510) 424-5905 (dane1@llnl.gov).
A Miniature Mass Spectrometer

Chemists and forensic scientists have been using mass spectrometers for decades to analyze a variety of sample materials. In the early 1980s, atomic physicists began to use the basic principles of the rectangular trap but are open-ended cylinders that fit inside a larger research unit. The combination of huge magnets and ultra-high vacuums, which can keep individual ions at rest, is what allows these ion traps to be so extraordinarily accurate.

The central detection device in research mass spectrometers is very small, which is what Dietrich and Keville found attractive. But the accompanying equipment fills a room. So, they developed the first truly hand-portable mass spectrometer, which fits into a small briefcase. It weighs just 12.3 kg (33 lb) and operates off a battery. Its accuracy is 1 in 1,000 (1:10^3), which is a long way from the accuracy of the large research units but sufficient for the uses planned for it.

Although this miniature mass spectrometer is still under development, it could have greater sensitivity and efficiency than conventional laboratory-based, single-pass mass spectrometers. In a single-pass unit, there are an ion source where the sampling material is ionized, an analyzer where the ions are separated in space according to their charge, mass, and velocity, and a detector that measures the number of ions analyzed. Some loss of ions as they move from region to region within this type of unit is inevitable.

Several innovations in our ion trap keep power consumption very low—to about half a watt. (That figure does not include the 20 W needed by the laptop computer that accompanies the device.) First, the permanent magnet is the most obvious power saver. Second, smaller is better. The smaller the trap is—that is, the closer the particles are to the detection electrodes—the greater the trap’s sensitivity and resolution are and the lower the electrical power consumption. Next, the unique design of the inlet valve is important in reducing energy consumption. In most mass spectrometers, gas is continually bled in, and large pumps are needed to maintain the vacuum in the spectrometer. This new miniature Piezo electric inlet valve lets in gas in small pulses, reducing vacuum pumping requirements. Finally, the vacuum pump itself is also a new power-saving design.

Uses in the Field

Research mass spectrometers used in laboratories are complex, bench-top instruments requiring a highly trained operator. Our inexpensive and portable mass spectrometer, in contrast, will find numerous applications outside of the laboratory. These units can act as air quality monitors in a closed or confined space, such as a factory where chemical weapons manufacture is suspected. Some day, with additional front-end filters or sensors, they could be used to indicate the presence of air-borne disease agents. In a home, office, or factory, they could be incorporated into feedback control loops and alarm systems to warn of hazardous conditions. A home sensor could monitor freon and radon as well as carbon monoxide, carbon dioxide, methane, propane, and other hydrocarbons. Law enforcement agencies could replace breathalyzers and drug- and explosives-sniffing dogs with these sensors. Testing drivers for alcohol use or sniffing out drugs could be performed remotely, which would mean increased safety for police officers and other officials.

Industries with critical process control functions could monitor for sensitive manufacturing byproducts such as chlorofluorocarbons, hydrazine, helium, nitrous oxide, sulfuric acid, and many others. Even small industrial accidents could be avoided with these sensors in place.
The electron beam gun described in this article was developed under a Cooperative Research and Development Agreement (CRADA) with American International Technologies Inc., Torrance, California. For further information about commercial applications, contact AIT at (310) 328-3484.

Smaller, Safer, Less Expensive

Depending on the power and features selected, the initial cost of a conventional system ranges from $300,000 to $2 million. A comparable sealed-tube unit and supporting equipment can generate 150 W of electron beam power. The new thin-film window, greatly reduces x-ray generation and improves worker safety. It is also more efficient because the reduced penetration depth of lower energy electrons is better matched to the thickness of the material being processed.

Operation at lower voltages, which is possible because of the thin-film window, greatly reduces x-ray generation and improves worker safety. It is also more efficient because the reduced penetration depth of lower energy electrons is better matched to the thickness of the material being processed.

Conventional systems require periodic replacement of the electron beam gun, which can generate 150 W of electron beam power. The sealed-tube electron beam gun can generate 150 W of electron beam power.

Expanding the Range of Uses

Currently, the primary commercial application for electron beam processing is curing polymers without using VOCs. Because electron beam polymerization does not produce VOCs and does not use the toxic photoinitiators used in ultraviolet light curing, it satisfies many of the existing and anticipated environmental restrictions on manufacturing processes. Electron beam processing is also used to sterilize medical supplies and food packaging materials that cannot tolerate sterilization by high temperature or chemicals. Electron beam sterilization of food has not gained widespread acceptance in the U.S., although its use is increasing in other countries.

The availability of a low-cost, self-contained source of high-energy electrons expands enormously the range of uses for electron beam radiation. Some examples include:• Fast, on-line chemical and elemental analysis. Electron beams induce visible, ultraviolet, and x-ray fluorescence whose spectrum identifies the elements in the irradiated sample. "Since this process is effectively instantaneous, it will likely find many applications both for on-line industrial monitoring as well as laboratory analysis," notes Myers.

• Injection of electrons into liquid sprays. The addition of electrons greatly improves spray effectiveness by reducing the average droplet size formed at the nozzle. Applications include pesticide spraying, turbine fuel injection, and internal combustion engine fuel injection.

• Destruction of toxic industrial compounds or cleanup of stack or automotive exhaust gases. Because electron beams are known to decompose both organic and inorganic compounds, they could be used as an economical method of waste treatment without incineration. The government might find this application useful for site cleanup and restoration programs. Another application of this new technology, to disassemble retired nuclear weapons, is under evaluation.

• Generation of ozone. Ozone is gaining popularity as a purifier for water supply systems, either instead of or in addition to chlorine. Ozone generation with electron beams is known to be approximately twice as energy efficient as conventional means using electrical discharge devices.

The electron beam gun described in this article was developed under a Cooperative Research and Development Agreement (CRADA) with American International Technologies Inc., Torrance, California. For further information about commercial applications, contact AIT at (310) 328-3484.

For further information contact Booth Myers (510) 422-7537 (myers5@llnl.gov) or Hao-Lin Chen (510) 422-6198 (chen4@llnl.gov).
The Genetic Contribution of Sperm: Healthy Baby or Not?

We have developed a powerful new tool using chromosome-specific DNA probes to study the causes of chromosomal abnormalities in sperm and their effects on embryonic development. The technique, fluorescence in situ hybridization (FISH), involves binding fluorescent labels to repetitive sequences of DNA in specific chromosomes. Its use in humans can provide valuable information on the sensitivity of sperm to environmental, occupational, and drug exposures as well as to other risk factors, including age and genetic variation. Such factors can result in chromosomal abnormalities that may be passed on to the embryo and fetus. Our recent studies show that a small fraction of human sperm from healthy males contains genetic defects, specifically abnormal numbers of chromosomes (aneuploidies). The proportion of such defective sperm appears to increase with age and smoking. These findings are supported by preliminary results on the frequency of aneuploid sperm of mice, which appear to be good models for studying induced aneuploidy. Treatment of men with Hodgkin’s disease using the chemotherapeutic drug NOVP induces a transient elevation in levels of aneuploid sperm. We are developing animal models to better understand how defective sperm affect the survival and development of the early embryo. We are also beginning to study fathers of children born with chromosomal defects, such as Klinefelter syndrome, to ascertain whether these men produce more aneuploid sperm than do fathers of healthy children.

Contact:
Andrew J. Wyrobek (510) 422-6296 (wyrobek1@llnl.gov).

Abstract

The Genetic Contribution of Sperm: Healthy Baby or Not?

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Contact:
Andrew J. Wyrobek (510) 422-6296 (wyrobek1@llnl.gov).
November/December 1995

Science & Technology Review

Commentary on Science and Technology Review November/December 1995

What Do You Think?

This issue of Science and Technology Review (formerly Energy and Technology Review) represents a modified approach to communicating the work of the Laboratory. The intent of our changes is to make this publication more interesting to a broader audience. Please give us your reactions to our changes by answering the questions in sections 1 and 2 below.

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Aerogels
Diode-Pumped Laser
Brightest Laser
Mini Mass Spectrometer
Electron Beam Gun

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A Light Fueled for Diode-Pumped, Solid-State Lasers
One of the World's Brightest Lasers
A Miniature Mass Spectrometer
More Efficient, Less Expensive Electron Beam Processing

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Science & Technology Review November/December 1995

Science & Technology Review

FAX Survey

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