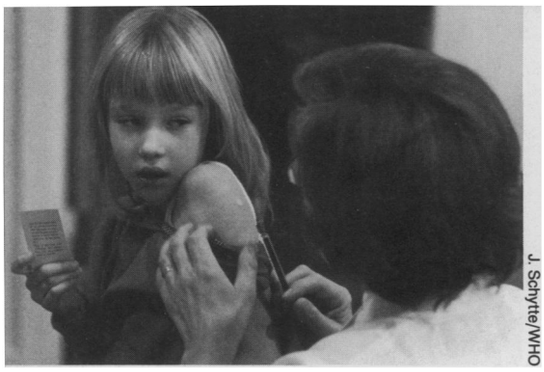
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A Shot in Time

The technology behind new vaccines

By DAMARIS CHRISTENSEN

Infectious diseases have ravaged the world's population for thousands of years. Prevention has a shorter history. In the 10th century B.C., the Chinese documented the practice of injecting pus from the smallpox pustules of a sick person into a cut on the hand of a healthy person. This induced form of smallpox was often less deadly — though no less infectious — than the original.

In 1796, English physician Edward Jenner noticed that dairymaids who caught cowpox — a similar disease found in cows — did not develop the more disfiguring, deadly smallpox. He soon developed the first safe, practical, preventive vaccine (from the Latin *vacca,* or cow) for smallpox and predicted the eradication of the disease. He may not have realized that it would take 180 years: The last case of wild smallpox was reported in Somalia in 1977.

Today, vaccines are among the safest, most cost-effective, and powerful tools for preventing disease. In 1990, the World Health Organization's Expanded Program on Immunization (EPI) reported that 80 percent of the world's children were immunized against measles, diphtheria, pertussis (whooping cough), tetanus, tuberculosis, and polio. Just 20 years ago, when the program started, less than 5 percent of children were fully immunized.

The very success of EPI carries the germ of failure. Because some infectious diseases have become so rare, parents in developed countries often neglect to take their children in time after time for vaccinations and booster shots: Complete immunization in the United States calls for a total of 17 shots and six visits in the first 15 months of life.

Although some 87 percent of U.S. children are fully immunized by the time they enter school, at age 2 only about 44 percent have received their full course of vaccinations. In the developing world, too, reaching children with additional doses poses a substantial problem.

Twenty percent of the world's children remain unimmunized. "Between 6 and 8 million children a year die of diseases that are theoretically preventable if we could either deliver the vaccines we have or make new vaccines to prevent these [infectious] diseases," says Col. Jerald C. Sadoff, director of the Division of Communicable Diseases and Immunology at the Walter Reed Army Institute of Research in Washington, D.C.

So researchers seek a 21st century "magic bullet." Working with a number of experimental techniques, they envision successfully developing a safe, effective, single-dose vaccine, given at birth, that can protect the world's children against a host of deadly ailments.

In 1990, the World Health Organization, the United Nations Children's Fund, the United Nations Development Fund, the Rockefeller Foundation, and the World Bank sponsored the Children's Vaccine Initiative (CVI) to pursue this goal.

The CVI wants to move technologies for new or better vaccines from the laboratory bench to the bush. It seeks affordable vaccines that require fewer doses, can be given earlier in life, can be combined in novel ways (reducing the number of shots and visits required), remain stable at tropical temperatures (reducing dependence on refrigeration), and are effective against new diseases.

"There's not a single aspect of this that would require a quantum leap in information or knowledge that we can see," says William H. Foege, director of the Task Force for Child Survival and Development at the Carter Center in Atlanta. "The question is whether we have the tenacity to go after it."

Vaccines mimic the organisms that cause disease, alerting the immune system that certain viruses or bacteria are enemy agents. Because of this advance warning system, when the "real" organism invades the body, the immune system can marshal a response before the disease has time to develop.

Ideally, the body will both make antibodies that bind to and disable the foreign invader (humoral immunity) and trigger white blood cells called T cells to attack cells in the body taken over by viruses (cellular immunity). Once the immune system's T cells and B cells, which make antibodies, are activated, some of them turn into memory cells. The more memory cells the body forms, the faster its response to a future infection.

Vaccines can trigger these responses in three ways. Some vaccines, such as those against smallpox, measles, and tuberculosis, contain genetically altered or weakened (attenuated) organisms that grow in the body after being administered but do not generally produce disease. Yet since the virus or bacterium is still alive, there is a small risk of developing the disease.

Whooping cough, cholera, and influenza vaccines are made of inactivated (killed) whole organisms or pieces of organisms. Because these organisms do not replicate inside the recipient, the vaccines confer only humoral immunity, which may be short-lived.

Finally, vaccines can be made against toxic products of these microorganisms. In diseases like tetanus, it is not the bacteria that kill, but the toxins they release into the bloodstream.

"It's quite clear that reducing the number of injections is incredibly important in increasing the effectiveness of delivery, both in terms of dollars and in terms of protection against disease," says Philip Russell of Johns Hopkins University in Baltimore, a special adviser to the Children's Vaccine Initiative. One of the easiest ways to do this, he says, is to develop more combination vaccines. Measles, mumps, and rubella vaccines are already delivered together, as are vaccines for diphtheria, pertussis, and tetanus (DPT).

DPT has been combined with a hepatitis B vaccine, an injectable polio vaccine, and a vaccine for *Haemophilus influenzae* type B, which causes meningitis, says Francis E. Andre, vice president of medical and scientific services at SmithKline Beecham Biologicals in Rixensart, Belgium. The pharmaceutical company is testing this combined vaccine in laboratory trials in Europe. But mixed vaccines may interfere with one another or reduce the stability of the vaccine, says Andre. This limits the number of vaccines that can be combined.

Alternatively, vaccine researchers can insert DNA that codes for surface antigens — substances recognized by the immune system as foreign — from many disease-causing microorganisms into one. When developed, these multivalent vector, or carrier, vaccines could act as a "Trojan horse," says Andre, and immunize against many diseases.

One of these diseases is malaria, which kills about 1 million people a year. "It's theoretically preventable," says Sadoff, "but the bug is getting ahead of our drugs." The protozoan that causes malaria has a complicated life cycle — it moves from mosquitoes to humans and

from human blood to the liver and back again. Because of this, he adds, it's been hard to make a vaccine.

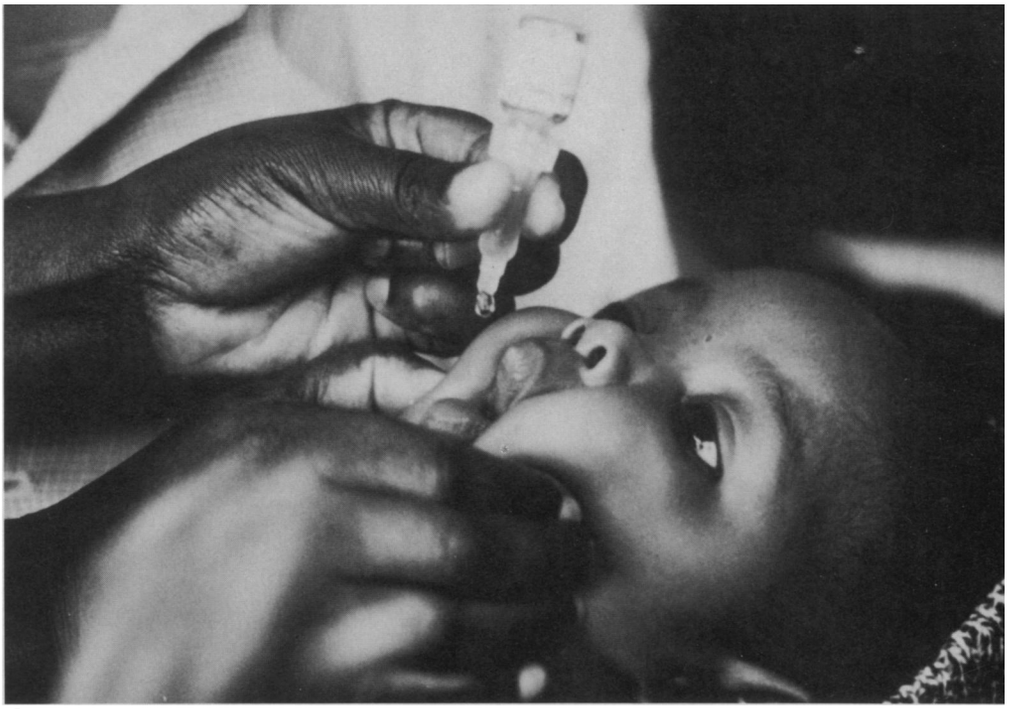
David Kaslow of the National Institute of Allergy and Infectious Diseases in Bethesda, Md., has recently developed a malaria vaccine that immunizes people against the stage of the protozoan transmitted to humans by mosquitoes. Human trials with this vaccine are scheduled to begin in Africa later this year.

Sadoff has combined antigens from each stage of the disease in a vaccinia (attenuated cowpox) virus. "This is the first time we've been able to make a combined vaccine [using] a single virus," Sadoff says. He notes that it also represents an organizational breakthrough, since each stage of the vaccine came from a different organization. The vaccine has been very effective in animal trials.

Another exciting development, says Sadoff, is injection of "naked" DNA. If one inserts genetic material from a pathogen directly into muscle tissue, a small proportion of this DNA is taken up by muscle cells. Those cells may make small amounts of antigens for up to 2 years. This ensures long-term cellular and antibody immunity, says Jeffrey B. Ulmer of Merck Research Laboratories in West Point, Pa. He has studied experimental naked-DNA vaccines for pneumonia and AIDS since 1990.

Whether naked DNA proves useful for vaccines will depend on whether it can be produced in large quantities and whether it has a good safety record, Ulmer adds.

"DNA is much more stable than live viruses. [DNA vaccines] have great potential and could very well revolutionize the way vaccines are made," he says.



E. Mandelmann/WHO

***A** Kenyan baby receives a protective dose of oral polio vaccine.*

The emerging technology of microspheres — antigens contained within shells made of the same plastic polymer used in resorbable sutures — may address several of the items on CVI's wish list.

The process of coating dried antigens with the polymer stabilizes the vaccine, thus extending its shelf life and reducing its dependence on refrigeration. Microspheres smaller than 10 micrometers are ingested by white blood cells called macrophages, which increases the body's immune response. Larger microspheres simply sit between cells. A mix of microsphere sizes might make possible a single injection of several vaccines that could be released at different stages to maximize immune response.

Researchers can tinker with the polymer coating to produce either a slow, steady release or bursts of vaccines that mimic additional injections. This may reduce the number of booster shots required for complete immunization, says Jacqueline D. Duncan, a pharmaceutical chemist at Secretech, a biotech company in Birmingham, Ala.

Microspheres can survive a trip through the stomach, which means that they can stimulate immune responses that can prevent the microorganism from ever getting into the blood. Oral vaccines are cheaper (no disposable needles) and easier to administer than injected vaccines and carry no risk of transmitting blood-borne diseases.

Duncan has already shown that once an initial injection of pneumonia or SIV (the primate version of HIV, the virus leading to AIDS) has primed the immune system in test animals, oral doses of microspheres can act as boosters. "I don't know of anyone who's having a great deal of success in people," she adds, "but many people are working on [it and] I'm sure we'll have that problem solved."

Adjuvants — chemicals that increase the immune reaction — may also reduce the number of booster shots required for vaccines, increase the immune response to oral vaccines, and extend the cold chain, or amount of time vaccines can go without refrigeration.

"We're seeing a burst of activity in a field with a strong background," says Carl R. Alving of the Walter Reed Army Institute of Research, who is developing adjuvants for malaria and HIV vaccines.

The adjuvants themselves are usually plant or bacterial substances placed in synthetic containers to deliver the vaccines to particular places in the body, Alving explains. "The only adjuvant currently licensed for use in humans is aluminum hydroxide," he says, "because it has such a long safety record. But it takes time, and there will be [new adjuvants] soon." His team of researchers, for example, has three clinical trials of different adjuvants under way in humans.

Many unimmunized children live in remote tropical areas. Extending the cold chain allows health workers to transport a vaccine farther without refrigeration.

Microspheres and better adjuvants both offer promise for the future, but consultant Karl Simpson and researchers at the Pasteur Institute in Paris have already achieved some success in lengthening the cold chain.

Of the six vaccines used by EPI, the oral polio vaccine is the least stable at tropical temperatures. The researchers reformulated the current polio vaccine with "heavy water" — water in which deuterium (a version of hydrogen with an extra neutron) replaces the hydrogen atoms.

Deuterium forms stronger bonds than hydrogen, says lead researcher Radu Crainic, and thus holds the viral genetic material and surrounding protein coat together more tightly. This stabilizes the polio vaccine so that it remains active at tropical temperatures for an additional 2 or 3 days, he notes, a technique that may work with other heat-sensitive vaccines.

Combination vaccines, multivalent vaccines, microsphere technology, and improved adjuvants have yet to reach their ultimate potential. Work on these technologies, however, has changed forever the vaccine development process.

"It's an enormous challenge, it's an enormous opportunity, and it has an enormous potential," says D.A. Henderson, former chief medical officer for the World Health Organization's Smallpox Eradication Program and now deputy assistant secretary for health and science at the Department of Health and Human Services. "It is, I think, the beginning of what promises to be a sea change in dealing with illness around the world. It's very exciting, with many more questions right now than answers." □