C V I F O R U M

The end of the CVI—reasons and lessons

Into the future with hi-tech vaccines

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In this issue

CHILDREN'S VACCINE INITIATIVE

All aboard, next time round

The Children's Vaccine Initiative (CVI) is a global coalition of organizations from the public, non-governmental and private sectors, including the vaccine industry, working together to maximize protection against infectious diseases through the development and wide utilization of safe, effective, easy-to-deliver vaccines. The CVI was launched at the World Summit for Children in 1990 and is cosponsored by the United Nations Children's Fund (UNICEF), the United Nations Development Programme (UNDP), the World Health Organization (WHO), the World Bank and the Rockefeller Foundation.

Cover photo: UNICEF/Jeremy Hartley

By a decision of its founding agencies, the Children's Vaccine Initiative is closing down.[1] This issue of the *CVI FORUM* is one of the last "products" of the CVI Secretariat, which will have ceased its activities by the end of this year. It is also *my* last opportunity, as one who has been involved in issues of vaccine development and delivery for many years and with the CVI Secretariat for the last five, to offer a personal perspective on the forces that have shaped the Initiative's fortunes and that ultimately led to its termination. In the broad view, of course, ideas and visions endure, even as institutions change under new leaders. And indeed, plans are under way to launch a new *Global Alliance for Vaccines and Immunization*, initially chaired by WHO Director-General Gro Harlem Brundtland. At this writing the mandate and *modus operandi* of the new entity are still being defined.

Roy Widdus, CVI Coordinator

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The early days of the CVI were marked by optimistic talk of visions and dreams—of harnessing the new power of science to bring into existence a few vaccines, or perhaps even a single vaccine, against all the most important diseases; of hastening the development and introduction of new vaccines; of devising new ways of bringing vaccination to the neglected children of the world. Since 1990, there has been progress towards some, but not all, of these goals. Polio eradication efforts, for example, have progressed significantly. Routine immunization, however, mostly with the vaccines for just six diseases, has remained

at 1990 levels and has become increasingly fragile in terms of financing and public confidence.

The CVI's existence over the closing decade of the 20th century has coincided with an explosion of activity and interest in vaccines (see the *Special Report* on pages 5-24). There has been a proliferation of ways in which vaccination *could* be improved and extended. There is also a wider recognition now of the need for a much greater global effort. I believe the CVI has played at least some part in these developments.

William Muraskin, the CVI's unofficial historian, concluded that, despite inter-agency tensions, "the CVI has helped change the international health scene in ways that can never be undone…[It] revolutionized the world of vaccines, which will never be the same."[2] And John La Montagne of the U.S. National Institute of Allergy & Infectious Diseases (NIAID), has called the CVI "a lubricant that keeps the world's vaccine machine—the immunization programmes, the research and development process, the financing mechanisms, and so on— working smoothly."[3]

Through its publications, among them the *CVI FORUM* and the 1998 *CVI Strategic Plan,* and through its regular discussion forum, the CVI Consultative Group, the Initiative, I am convinced, created and nurtured the very concept of an integrated vaccine community. Through these instruments, it fostered "inclusion," giving voice and attention to many hitherto unheard members of that community—to managers of vaccination programmes in the poorest countries faced with sometimes impossible political, social and economic conditions; to researchers seeking a humanitarian outlet for their scientific know-how; to "local" producers trying to provide quality vaccines in developing countries; to industry leaders wanting to break out of the venal mould into which holier-than-thou public-sector agencies have traditionally cast them; and to advocates for children, whose rights of access to vaccination are still being flouted in too many countries.

There are other contributions which the collaborators in the CVI and the CVI Secretariat staff can be proud of. To mention only a few:

❍ Creating the machinery for examining the quality and supply of vaccines produced in developing countries and for doing something about the deficiencies in this neglected area.

❍ Bringing home to the world the need—not as an option but as a duty of the vaccine community—to introduce rapidly into developing countries the many emerging new vaccines. The CVI also prepared and distributed step-by-step agendas for fulfilling this duty.

❍ Creating the foundations of a public-private sector partnership. Industry, unfortunately, never achieved the formal, legal status of a full partner, but not for lack of CVI Secretariat efforts and industry inclination.

❍ Calling attention to other unaddressed needs in vaccine development and delivery: harmonization of international regulatory requirements, mechanisms for sustainable financing, better communication of the health and economic benefits of vaccination, greatly expanded mobilization of resources, closer attention to under-utilized vaccines and neglected diseases, better ways of determining priorities and formulating policy for vaccines and vaccination, and new mechanisms for targeting assistance to countries in greatest need.

These contributions were made despite several flaws in the CVI's "machinery"—some dating from its outset—that diminished its potential:

❍ As documented by Mr Muraskin, the "desires" of international agencies regarding the role of the CVI differed, their level of interest fluctuated and to differing degrees they were sometimes unable to put aside their parochial, organizational interests. Throughout the entire history of the CVI, this ambivalence made agreement on the CVI's role and activities difficult to achieve, which in turn adversely affected the participation of some bilateral agencies.

❍ The CVI Secretariat was housed—physically and legally—within the WHO, which is a public-sector agency and cannot formally integrate private-sector entities. This

"The CVI has helped change the international health scene in ways that can never be undone."

led to confusion over the CVI's identity and prevented the creation of a true coalition of equal partners that included the private sector.

❍ Some agencies failed to recognize the benefits of a long-term vision that called for immediate actions likely to yield great benefits for coming generations. They therefore gave low priority to these actions.

❍ Despite the widely recognized need for better co-ordination of inputs into country activities, the decision was taken to remove these "operational" activities from the CVI's mandate.[4]

❍ The CVI's income, which rarely exceeded U.S. $2 million per year, consistently fell short of its approved budget.

The overall result of these flaws was a CVI with wings too clipped to allow it to take off and with co-pilots arguing about whether it should try to fly and what its destination should be.

Yet, the CVI *did* make its mark. It provided, for the first time, a "space" where those willing to do so could put aside their organizational allegiances and speak their minds as individuals. A place where new partnerships and collaborations could be initiated. A space from which industry CEOs could go beyond budget lines and express their humanitarian concerns. A space from which the participants in the CVI coalition could highlight in public view the disparity between the growing scientific opportunities and the unmet needs of the world's children.

And therein, perhaps, lies a reason for the CVI's termination. Its constant calls for urgent action cast into stark relief the many omissions in the response. The CVI and its Secretariat had the capacity to identify the problems and their remedies but not the structure nor the backing to bring about the solutions.

Today, the new coalition emerging from the ashes of the CVI is preparing to face these unmet needs. It is doing so from a richer base of scientific opportunity and vaccination experience than the CVI had at its birth. Although I cannot help regretting that the CVI was not re-engineered to help solve the problems it had highlighted, I hope that those shaping the new coalition will have learned a lesson from the CVI "experience."

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The lesson, perhaps, is that to move forward into the future, a future where vaccination is able to fulfil its life-saving potential, the vehicle must be right and the team steering it must be right. But more important: everyone must be on board. Not just physically, financially and administratively, but philosophically, putting the needs of children and adults for vaccination and other health services above all other considerations.

Roy Widdus,
Coordinator,
The Children's Vaccine Initiative (CVI)

Notes

1 In March 1998, the World Bank hosted a meeting *(Vaccine Development and Delivery: Leadership for the 21st Century)* of public and private-sector leaders. Over the next 12 months, a working group prepared background documents, incorporating the findings of a wide-ranging consultation of experts, for a follow-up meeting held in March 1999 in Bellagio, Italy. At that meeting, the CVI's co-sponsors decided to end the CVI and create a new entity.

2 W. Muraskin, *The Politics of International Health*, State University of New York Press, Albany, NY, USA, 1998.

3 *CVI FORUM* No. 14, June 1997.

4 By the Meeting of Interested Parties, the CVI's top management body, held in November, 1994, in Amsterdam.

A plethora of hi-tech vaccines—genetic, edible, sugar glass, and more

Gee-whiz technology has come to vaccines, firing the imagination of the media, the general public and even much of the normally sceptical scientific community. This decade, which began with the birth of the CVI and is ending with its demise (see Editorial, *pages 2-4), has witnessed an efflorescence of scientific discovery and exploration. This* CVI FORUM *report looks at a necessarily limited and somewhat arbitrary sampling of products emerging from recent research. The four approaches reviewed here—DNA vaccines, transgenic plant vaccines, sugar glass vaccines and skin patch vaccines—give a taste of the possibilities and challenges offered by today's science.*

One thing just about all public health pundits agree on is that vaccination is the most reliable, least expensive way of making a community healthy and keeping it that way. In the U.S. alone, vaccines have all but wiped out a whole constellation of infections—diphtheria, measles, mumps, pertussis, polio, rubella, tetanus, and meningitis due to Hib (*Haemophilus influenzae* type b)—that in pre-vaccine days were producing nearly 2 million episodes of disease a year. Other developed countries show a similar picture. World-wide, vaccines are reaching about 80% of the world's children and saving every year three million from death by infection, as well as preventing blindness, paralysis and mental disability in a further 750,000.

Why, then, with vaccination such bad news for bugs, with over 100 vaccines available today in different forms and formulations against some 40 infections, and with four-fifths of the world's children receiving immunization against the "basic" childhood infections, are about 12 million kids still dying every year from infectious diseases?

Why are about 12 million kids still dying every year from infectious diseases?

UNICEF/Roger Lemoyne

Vaccines of the future should be easier to administer to people living in harsh conditions, as these Rwandan refugees.

About two-thirds of these deaths are caused by infections for which satisfactory vaccines are not yet available.

Table 1. Death toll from infections by availability of a satisfactory vaccine

diseases	deaths (000)[a]	%
no satisfactory vaccine available		
AIDS	2285	30.39
Tuberculosis	1498	19.92
Malaria	1110	14.76
Pneumococcus	1100	14.63
Shigella	600	7.98
Enterotoxigenic *E. coli*	500	6.65
Respiratory syncytial virus[b]	160	2.13
Schistosomiasis[c]	150	1.99
Leishmaniasis	42	0.56
Trypanosomiasis	40	0.53
Chagas disease	17	0.23
Dengue	15	0.20
Leprosy	2	0.03
total deaths	*7519*	100.00
a satisfactory vaccine available		
Hepatitis B	1000	24.55
Measles	888	21.80
Rotavirus	800	19.64
Haemophilus influenzae type b	500	12.27
Tetanus	410	10.06
Pertussis	346	8.49
Cholera	120	2.95
Diphtheria	5	0.12
Japanese encephalitis	3	0.07
Poliomyelitis	2	0.05
total deaths	*4074*	100.00
GRAND TOTAL	11593	

a Estimated, World Health Report, WHO, 1999
b The Jordan Report, NIAID, 1998
c R. Bergquist, WHO, personal communication

satisfactory vaccines are *not* yet available (see Table 1). Some new vaccines are likely to come to market in the next year or so (against pneumococcal disease, for example). Others still face formidable scientific obstacles (HIV/AIDS, malaria, respiratory syncytial viral infection, shigellosis), obstacles compounded in some cases by tepid political commitment and low industrial incentive for vaccines mainly of use in countries too poor to pay for them.

For the four million deaths from infections for which vaccines do exist the picture is more complex. Some of the vaccines currently available (e.g. against tuberculosis and cholera) are clearly not effective enough to make a serious dent in mortality figures. Some cost too much for the poorest countries to buy (hepatitis B and Hib). Some of the big killer infections (Hib, for example) just don't loom large enough in the public or political eye in many of the countries where they could reduce child mortality. And finally, that 80% global vaccine coverage rate is just an average—in many parts of the world, mainly in some African countries, the basic childhood vaccines are not getting to even 50% of children: the reasons are many—crippled or non-existent health services in areas ravaged by war or economic crisis and decision-making on health matters skewed towards treatment rather than prevention, to name two.

Cutting the toll of those "needless" 12 million deaths means addressing the need for new vaccines and for better versions of available vaccines that might, among other things, make it easier to vaccinate more children.

The last decade has seen an explosion of activity in the vaccine research and development community, fuelled by three trends: a growing realization among health authorities, at least in the industrialized world, of the value of vaccination; an increasingly high ranking given to vaccination and vaccines by international humanitarian, health and financial agencies; and enormous strides in the science and technology that underpins the development of new and better vaccines and that has spawned a new breed of small biotech companies.

Total funding worldwide for vaccine R&D from all major sources, public and private, now stands at around $1 billion, almost double its level a decade ago (vs. $50 billion for other pharmaceutical products!). Funding by one of the world's biggest supporters of vaccine research, the U.S. National Institutes of Health, will have almost tripled by the end of the century for research on vaccines, including HIV/AIDS vaccines, up from $158 million for fiscal year 1990.[1] Nor has the vaccine industry been sleeping through the decade: total sales revenue from vaccines world-wide now

UNICEF/Roger Lemoyne

Lining up for the traditional jab in a Mozambique health centre. The new vaccines, which hold the promise of oral or nasal administration, could make vaccination a painless procedure.

stands at about $6 billion,[2] up from about $2 billion ten years ago, with firms still spending at least 15% of their revenue on vaccine R&D. And while the number of major vaccine manufacturers has shrunk in recent years to a mere handful—largely as a result of mergers—the number of biotech firms with a vaccine-dominated portfolio has blossomed over the decade from a dozen or so to over 70—a reflection of the growing application of molecular genetics and other basic science disciplines to innovative vaccine development. (Interestingly, *Vaccine*, a leading vaccine research journal, increased its number of pages seven-fold between 1984 and 1998).

John La Montagne, Deputy Director of the National Institute of Allergy & Infectious Diseases, part of the U.S. National Institutes of Health, says: "The explosion of interest in vaccines in both private and public sectors has been driven by a better understanding of the immune system and its processes. We have a better idea now how antigens are presented to the immune system and how that affects protective immune responses. And we are probing to ever-greater depths into the mechanisms pathogens use to infect us and cause disease. Genomic research has sequenced some 20 organisms and sequencing is currently in progress for another 47 organisms—that is an incredible achievement and vaccine research is benefiting from it."

A recent analysis of the economics of vaccine innovation found "the situation in vaccines…much more promising today than it was a decade ago. There has been a renaissance in terms of innovative effort…There is an impressive pipeline of R&D projects involving new vaccines." [3] There are now in fact nearly 500 candidate vaccines in that pipeline[4] and the principle of vaccination is beginning to extend its reach to diseases not traditionally thought of as infections—some forms of blindness, cervical cancer, gastric ulcer, coronary artery disease, Whipple's disease, and the list is growing. Even multiple sclerosis, rheumatoid arthritis, schizophrenia, diabetes and peanut allergy, to name only a few examples of *non-infectious* conditions, are now the targets of vaccine candidates. The toolbox of vaccine accessories—adjuvants, vectors (synthetic or live), delivery devices (injectors, guns, aerosols, etc.), formulation technology (dryers, nebulizers, etc.)—is bursting at the hinges, although only a few products have so far emerged into actual use.

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The "new generation" technologies seek to make vaccines that are more easily and efficiently administered; that are as effective or more effective for longer and in less frequent doses; that can be produced more efficiently to equal or greater standards of quality; and that are more stable in a wider range of environmental conditions.

DNA vaccines provoke immune responses to		
viruses	cytomegalovirus	canine parvovirus
	dengue	canine distemper virus
	Ebola	coxsackievirus
	herpes	foot-and-mouth disease virus
	influenza	feline immunodeficiency virus
	Japanese encephalitis	hantavirus
	Marburg	HTLV-1
	measles	Sendai virus
	rabies	
	rotavirus	
	rubella	
	simian HIV (SHIV)	
bacteria	*Bacillus anthracis* (anthrax)	enterotoxigenic *E. coli*
	Borrelia burgdorferi (Lyme disease)	*Yersinia enterocolitica*
	Chlamydia	*Mycobacterium* species
	Clostridium tetani (tetanus)	
	Mycobacterium tuberculosis (TB)	
	Mycoplasma	
	Shigella	
fungi	*Coccidioides immitis*	
parasites	*Leishmania*	*Cowdria ruminantium*
	Onchocerca (river blindness)	*Cryptosporidium parvum*
	Plasmodia (malaria)	*Tænia crassiceps*
	schistosomes	*Trypanosoma cruzi*
Immune responses to DNA vaccines seen in		
	birds	cats
	chimpanzees	goats
	cows	guinea-pigs
	dolphins	
	ferrets	
	fish	
	mice	
	monkeys	
	pigs	
	rabbits	
	rats	
DNA vaccines provoke protective immune responses against		
	anthrax	canine parvovirus disease
	cervical cancer	canine distemper
	coccidiodomycosis	hand-foot-and-mouth disease
	cytomegalovirus disease	*Yersinia enterocolitica* infection
	dengue	feline immunodeficiency
	Ebola haemorrhagic fever	*Cowdria ruminantium* disease
	hepatitis B	*Cryptosporidium parvum* infection
	hepatitis C	cysticercosis
	herpes simplex	sleeping sickness
	HIV/AIDS	
	influenza	
	Japanese encephalitis	
	leishmaniasis	
	Lyme disease	
	malaria	
	multiple sclerosis	
	peanut allergy	
	rabies	
	rotavirus disease	
	rubella	
	tick-borne encephalitis	
	tuberculosis	

Table 2: Naked DNA-a summary of animal research to date

Gustav Nossal, chairman of the CVI's Scientific Advisory Group of Experts and Professor Emeritus of the University of Melbourne's Department of Pathology, in Victoria, Australia, speaks of a "ferment of research [that] has brought forward what could almost be termed a surfeit of riches."[5]

Generally speaking, the most promising of the "new generation" technologies—including the four described below—seek to make vaccines that are more easily and efficiently administered (by mucosal routes—mouth, nose, gut, etc.); that are as effective or more effective for longer and in less frequent doses; that can be produced more efficiently to equal or greater standards of quality (at the lowest possible cost); and that are more stable in a wider range of environmental conditions (and so require less complex logistics, such as the cold chain, than some current vaccines).

DNA vaccines

"Arguably the most powerful development of all, DNA vaccines have made their explosive entry, possibly signalling a revolution in vaccinology."[5]

Sir Gustav's statement is correct: the U.S. researchers[6] who in 1989 discovered quite by chance during a gene therapy lab experiment that a gene inserted directly into a mammalian cell—i.e. without using a live vector, like a virus—could induce the cell to manufacture (*express*) the protein coded for by the gene, were flabbergasted. "We tried it again, and it worked," recalls Jon Wolff, who headed the research team at the University of Wisconsin in Madison, USA. "By the fourth or fifth time, we knew we were onto something big." Step one.

Three years later, a team headed by Stephen Johnston at the University of Texas, Dallas, reported that the "naked DNA" technique, as it came to be called, could not only trigger the production of foreign proteins in recipient animals but that these proteins could produce an immune response in the animals.[7] Step two.

Before Dr Wolff's discovery—since the early 1960s in fact—there had been several attempts to transfer genes directly into living cells, but with only modest success. In the 1980s, a number of groups in the U.S. did basic and animal research on naked DNA for vaccination purposes, among them Harriet Robinson at the University of Massachusetts, Worcester, David Weiner at the Wistar Institute in Philadelphia, Pennsylvania, and Dr Johnston's group in Dallas, Texas. But somehow the time wasn't ripe. "It took us a year to get published," recalls Dr Johnston, "because no-one thought the technology was useful. A *Nature* reviewer in 1991 referred to it as 'cute at best'."

Over the four years after publication of Dr Wolff's report in 1990, researchers working in industry and academia showed in animals that the foreign protein expressed by cells transfected with naked DNA could provoke a *protective* immune response. Step three.

The DNA was injected or shot into muscle tissue by a "gene-gun" or blown into skin as a powder. The immune response occurred in a broad cross-section of the animal kingdom—from mice to dolphins and birds. It showed a degree of protection in animals against viruses, bacteria, mycobacteria, fungi and parasites (see Table 2). Experimental DNA vaccines have also shown protection in animals against cancer (T-cell lymphoma, B-cell lymphoma and renal carcinoma), auto-immune disease (multiple sclerosis) and allergies (peanut allergy).

Excitement and enthusiasm spread quickly through the vaccine community, eliciting praise, as well as scepticism from those who doubted whether DNA-transfected host cells could produce enough foreign protein that would interact in the right way with the body's immune cells to provoke a protective immune response. "Some scientists considered it black magic and used words like 'cold fusion' to dismiss it," says Margaret Liu, an early gene vaccine adept, now Vice-President for Vaccines and Gene Therapy Research at Chiron Technologies at Emeryville, California, USA.

The animal studies dispelled much of the scepticism. Immunologists were particularly excited about the ability of DNA vaccines to provoke a broad immune response comprising not only antibodies (*humoral immunity*) but also T cells (*cell-mediated*

"The use of DNA, a nonliving agent, to raise cytolytic T cells represents a milestone in vaccinology."

immunity), especially cytotoxic (also known as "cytolytic") T cells (CTL). Dr Liu calls this ability "the immunologist's grail."[8] Strong CTL responses were previously associated only with "live" vaccines, like the measles or oral polio or tuberculosis (BCG) vaccine. "The use of DNA, a nonliving agent, to raise cytolytic T cells represents a milestone in vaccinology," the American Academy of Microbiology wrote.[9]

Experimental naked DNA vaccines also appeared to offer, at least in animals, the promise of long-lasting protection, stability (i.e. little loss of potency over time and in extreme environmental conditions) and simplicity of production—benefits that underscored their potential for use in developing countries. Unlike live viral or bacterial vaccines, which can revert to an infective form, although do so very rarely, DNA vaccines cannot cause an infection. Moreover, because a DNA vaccine consists of a protein antigen produced by the vaccine recipient's own cells using the same processes used by these cells when they are invaded by infectious agents, the antigen and the response may be more "natural" (*native*) than in the case of antigens produced by traditional vaccine production methods (e.g. viruses grown in animal cells).

A big attraction of DNA vaccines is the ease with which they can be made to carry multiple genes coding for a whole range of vaccinating antigens.

Gene vaccination is also proving useful as a research tool: DNA vaccine technology can be used to screen whole genomes of pathogenic organisms to find the best gene for a desired immune response. Dr Johnston's group in Dallas has created DNA plasmid "expression libraries" for the genes of whole sections of a pathogen's entire genome and administers them to laboratory animals. "We can literally shoot as many as 27,000 different plasmids into a mouse at once," he says, "and get an immune response to an individual plasmid in that group." Libraries that don't confer protection to a given infection are cast aside until the few genes that do show protection are identified.

For Stephen Hoffman, too, who heads the U.S. Naval Medical Research Center's malaria programme in Rockville, Maryland, a big attraction of DNA vaccines is the ease with which they can be made to carry multiple genes coding for a whole range of vaccinating antigens. Dr Hoffman and his team, together with an international roster of collaborating groups, have built DNA vaccines consisting of five, ten, even 15 genes in different plasmids encoding different antigens from different life-cycle stages of the malaria parasite. "To deal with that complexity," he says, "is inordinately difficult with common protein vaccines. The real draw of the DNA technology is its capacity to deal with complexity, the complexity of a malaria parasite with its 6,000 or so genes and the complexity of a child, say, with malaria who may be infected with five to ten different strains of the *Plasmodium falciparum* parasite causing the malaria."

Other vaccine researchers and developers pin a lot of hopes on the amenability of the DNA technology to a wide range of production facilities. Ripley Ballou, up to recently director of immunology at the Walter Reed Army Institute of Research in Washington, D.C., and now with the biotech firm MedImmune of Gaithersburg, Maryland, believes "a major reason for the interest in DNA vaccines is the generic delivery system on which it is based: production is standard and more generic than for any other vaccine technology and so could theoretically be readily applied in developing countries. The big question now is: does it work in people?"

At this writing, there are about 40 DNA candidate vaccines,[10] designed to protect against about 20 different conditions, from anthrax to allergy (see Table 2). Five are in human trials, all still being tested for immunogenicity and safety in the earliest (Phase I) stage. They are designed to protect against hepatitis B, herpes simplex (types 1 and 2), HIV (2 candidates), influenza and malaria.

With the exception perhaps of the malaria candidate vaccine, which induced CTLs in humans, the results of clinical trials have so far not lived up to the early optimism. "DNA vaccines," says Dr Liu, "have been very potent in animals, giving robust immune responses. Everybody, so to speak, was getting protection in some animal model or another. But the clinical trials have been disappointing, either because the level of the response was inadequate or because too high doses of DNA had to be used to get a reasonably adequate response."

One problem has been inefficient uptake of the plasmid DNA by host cells: only a small amount of the dose administered actually ends up inside cells. Many researchers faithful to the method, including Drs. Liu and Hoffman, have returned to their lab benches to improve the technology and are working on several approaches:

❍ Instead of a plasmid, researchers use a virus to carry the microbial DNA into host cells. Among viruses they are manipulating for this purpose is an RNA bird virus, the Sindbis virus, of the alphavirus family, which can infect human cells without causing disease. It can also target "professional" immune cells (*antigen-presenting cells*), which are presumably the cells best able to provoke a strong immune response when transfected with the foreign DNA. Dr Liu's team at Chiron has increased the potency of a DNA plasmid up to 1,000-fold using this alphavirus system.

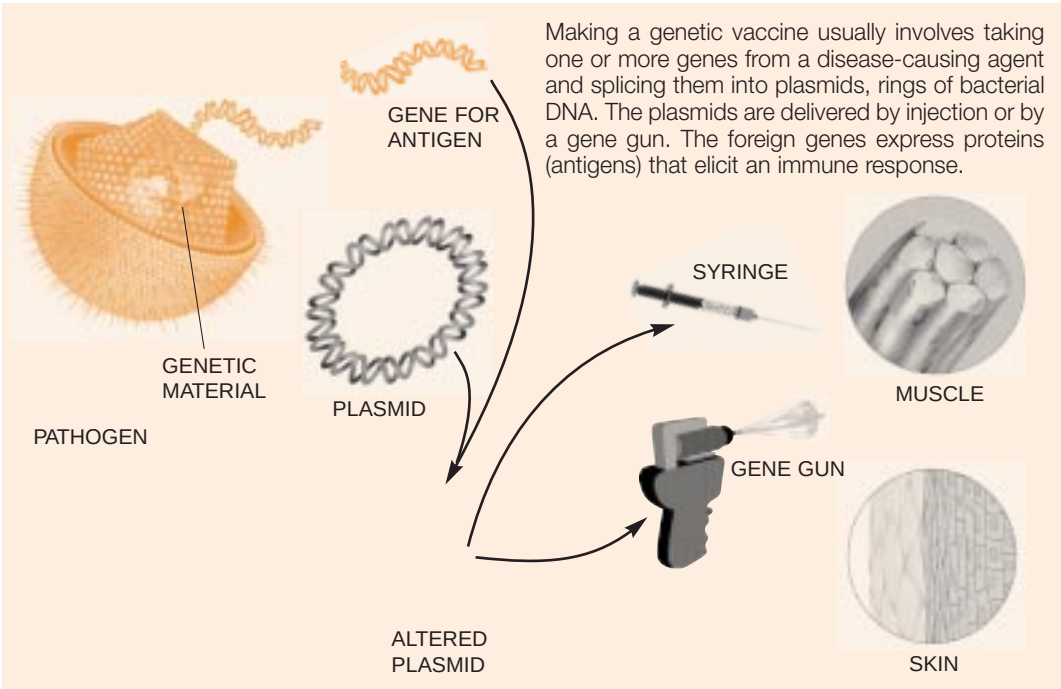
❍ Some scientists are even putting plasmids back into bacteria. Among bacterial plasmid vectors being worked on are genetically disabled strains of *Shigella*, a cause of diarrhoeal disease. *Shigella* normally invade humans via the intestinal tract, so the *Shigella*-plasmid vaccine might be administered orally, producing protective immunity not only to the organism corresponding to the foreign microbial DNA but also to itself. *Salmonella*, the cause of typhoid fever, and *Listeria,* a cause of meningitis and septicaemia, are also being tried out as DNA plasmid vectors.

❍ Wrapping the DNA plasmid in a biodegradable polymer will protect it from damage or destruction by the acidic gastrointestinal environment when given orally: last year U.S. researchers observed in mice a generalized (systemic) and a local (gastrointestinal mucosal) immune response to a rotavirus antigen delivered this way.[11]

❍ Animal studies are under way on DNA mixed with alum, the traditional vaccine adjuvant, which apparently can increase the immune response to the protein encoded by the DNA.

❍ Cytokines are released by immune cells and "help" the immune system combat an infectious agent. Splicing a cytokine gene into a DNA plasmid so that it is expressed in host cells along with the microbial antigen is an approach being investigated by a few research groups. Among them is Dr Hoffman's team, which is testing the insertion of the gene for a cytokine, granulocyte-macrophage colony stimulating factor (GM-CSF), into a malaria DNA plasmid vaccine: they give a mixture of plasmids encoding malaria antigens and GM-CSF to prime the immune system, followed several

"The clinical trials have been disappointing, either because the level of the response was inadequate or because too high doses of DNA had to be used to get a reasonably adequate response."



Making a genetic vaccine usually involves taking one or more genes from a disease-causing agent and splicing them into plasmids, rings of bacterial DNA. The plasmids are delivered by injection or by a gene gun. The foreign genes express proteins (antigens) that elicit an immune response.

From Genetic Vaccines by D.B. Weiner and R. C. Kennedy, Scientific American, July 1999

months later by a booster dose of a vaccine using a recombinant avian virus as a vector for the malaria antigens. Researchers are also testing this "prime-boost" approach in candidate vaccines against HIV/AIDS and cytomegalovirus infection.

❍ To augment the yield of protein antigen produced by DNA-transfected host cells, some groups, including Dr Hoffman's, are tinkering with the plasmid DNA sequences (codons) coding for the amino acid triplets that make up the protein, so that they function in a more "human" manner and are more efficient at making the foreign protein.

❍ Application of an electric field to a living cell causes a transient permeability in its outer membrane. Currently in Phase III clinical trials for drug delivery in cancer patients, the method, known as "electroporation," uses needles inserted into the skin to deliver an electrical pulse. In animal studies currently under way in the U.S. a DNA vaccine injected in conjunction with electroporation produced at least a 10-fold increase in the antibody response to a DNA vaccine, compared with non-electrostimulated control animals.

❍ Researchers are also seeing enhanced immune responses by fashioning the DNA molecule into a particle that antigen-presenting cells are known to have an affinity for. Others are applying DNA plasmids to the skin by lotion or cream or skin patch.

❍ Some naked-DNA experts believe that intramuscular injection is not the best route of delivery for DNA vaccines. Dr Johnston at the University of Texas notes that "most people in the field realize now that the DNA should be directed to the dendritic cells in the skin and mucosa" (see page 21).

One might think that having researchers back in their labs pottering with a technique that is already in clinical trials might seriously hamper its development. But Dr Hoffman is not fazed. "Sure," he says, "we're going back to our benches and putting our trials on hold while we incorporate the very latest developments into the technology.

"Sure, we're going back to our benches and putting our trials on hold while we incorporate the very latest developments into the technology. But with this technology, it's so simple that one can do that relatively easily and quickly."

Gene vaccines—how safe?

The public knee-jerk reflex to the mere mention of gene biotechnology is at best a raised eyebrow, at worst, panic. Perhaps because of this reflex and also because of the great potential DNA vaccines have to become extremely useful public health tools, a lot of research has gone into their safety.

Some lingering questions on this topic:

❍ Could antibodies to a DNA vaccine recognize the vaccine recipient's own DNA and cause autoimmune disease? *Not likely, say researchers: purified DNA given to animals does not readily produce an antibody response and DNA vaccines administered to animals have induced little or no antibodies to the DNA. Don't forget, these vaccines are designed to produce an immune response not to the DNA, but to the protein produced by the cells penetrated (transfected) by the DNA. And besides, most people do carry antibodies against DNA—from bacteria, for example—and are exposed to DNA all the time in bacteria and food, and don't seem to suffer ill-effects.*

❍ Do we know fully how DNA vaccines work? *Quite a lot is now known about how they work. Note that we still don't understand fully how a number of commonly used vaccines work, such as those against measles, pertussis and rotavirus, to mention only three.*

❍ Unlike the more traditional vaccines, DNA vaccines tend to hang around for a long time (up to six months in mice) in host cells: this is fine for immunization purposes, but could prolonged immune stimulation not cause chronic inflammation or an auto-immune reaction? *Just how long the DNA hangs around is not altogether clear but safety studies in animals have shown no ill-effects of this type.*

❍ Could the DNA vaccine integrate with the host's own DNA in ways that might not be in the host's best interests, like turning on a cancer gene *(oncogene)* or turning off a (*repressor*) gene that holds an oncogene in check? *Painstaking studies looking for integration in animals given DNA vaccines have not found it to date.*

Three steps to making and using DNA vaccines

If you want to set up a do-it-yourself gene vaccine production unit, the best advice is "don't." Even first-generation DNA vaccines take a considerable amount of hi-tech savvy. The following "primer" may give you, though, an idea of some of the basic principles underlying the technology (see diagram, page 11):

❍ Take an easily manipulated bacterium like *Escherichia coli.* Reach inside its cell and gently pull out one of its plasmids (that's a circular DNA molecule probably minding its own business in a corner of the cell—the bacterium uses it, among other things, to transfer its genes to other bacteria).

❍ From the genes of the microbe against which you want to make a vaccine—influenza virus, malaria parasite, whatever—take one that codes for a protein molecule (antigen) known to provoke a strong immune response in people (or in the animals you're using for your experiments) and insert it into the plasmid. Don't forget to add an "ignition" system for your foreign gene—namely, the sequences to start it up *(promoter)* and to stop it *(polyA).* These have to come from a eukaryotic organism, like a plant or an animal, or from a virus, to be recognized by the mammalian cell machinery.

❍ Vaccinate by injecting the DNA plasmid in a saline solution into muscle; or by shooting tiny gold beads coated with the DNA plasmid into the skin with a "gene gun;" or by blowing into the skin a stream of liquid or powdered DNA plasmid at supersonic velocity with a jet injector; or by spraying the DNA vaccine into the nostrils with an aerosol device; or by wrapping the DNA in a biodegradable microcapsule and administering it orally; or by applying it on

With edible vaccines the plants not only manufacture the antigen but also deliver it into the host.

But with *this* technology, it's so simple that one can do that relatively easily and quickly: we're talking about a delay of a few months, compared with years for most other vaccine development approaches."

Dr La Montagne at the NIAID is convinced that "the whole area of DNA vaccines has an enormous potential if it can be brought to fruition and if some lingering safety concerns are dispelled." (See Box, page 12)

Long-time expert on DNA vaccines, Harriet Robinson, now chief of microbiology and immunology at the Yerkes Primate Research Center in Atlanta, Georgia, USA, has little doubt that DNA vaccines "could well be the (major) method for the development of new vaccines for the next century."

Edible plant vaccines

In the mid-1980s, just a few years before DNA vaccine technology broke upon the scene, U.S. molecular biologists Roy Curtiss III and Guy Gardineau[12] were chatting in a car about recent scientific reports that foreign DNA could be inserted into the genomes of plants—notably, tobacco and petunia[13,14]—and that the resulting *transgenic* plants successfully expressed the foreign DNA. Why not use the same approach, the two friends mused, to coax *edible* plants into expressing vaccine antigens in their leaves or roots, that could be eaten by people and thus offer a more pleasurable vaccination experience than the traditional jab? Thus was conceived the notion of edible vaccines.

Although the concept resembles that of DNA vaccines, it differs in several respects. Whereas, with DNA vaccines the gene for the vaccinating antigen is inserted directly into host cells without a living vector, with edible vaccines the plants not only *manufacture* the antigen but also *deliver* it into the host.

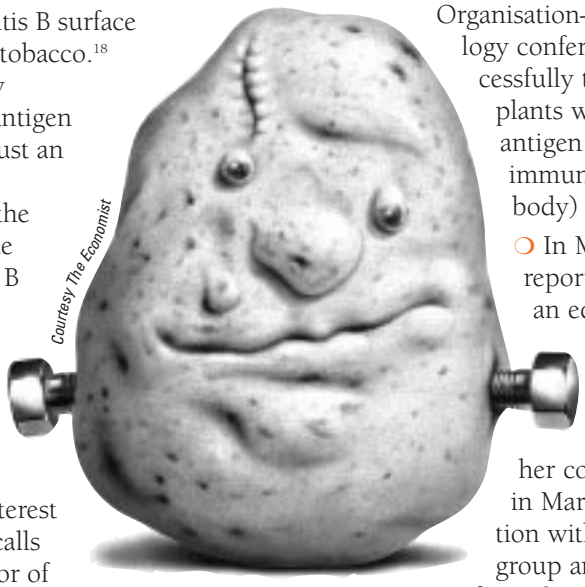
Courtesy The New Yorker

"We were taken by surprise at the tremendous media interest in plant vaccines. The idea of an edible vaccine really titillated the popular imagination."

Moreover, the "classical" edible vaccine technique involves not one DNA transfer step—as with DNA vaccines—but two, and requires two bacterial plasmids (see Box, page 13).

Over the past decade and a half, at least 50 plant foods have been genetically "enriched" with a variety of virtues, both agricultural and commercial. The technology is well-established.

Thanks to the work of Charles Arntzen and his colleagues at the Boyce Thompson Institute for Plant Research (BTI) in Ithaca, New York,[15,16,17] who nursed the concept through its infancy and adolescence, and have tirelessly proclaimed its potential to the world at large, edible vaccines very quickly gained a foothold in the hallowed halls of vaccine science. In 1992, the group published the first report of successful expression of a hepatitis B surface antigen in transgenic tobacco.[18] Three years later, they announced that this antigen could produce as robust an antibody and cellular immune response as the commercially available yeast-grown hepatitis B vaccine.[19]



Courtesy The Economist

At this point, the press began to "smell" a story. "We were taken by surprise at the tremendous media interest in plant vaccines," recalls Myron Levine, Director of the University of Maryland's Center for Vaccine Development (CVD) in the United States. "The idea of an edible vaccine really titillated the popular imagination." Nor was industry blind to its promise: there are now at least five biotechnology companies for which the development of edible vaccines is their sole or major aim in business.

Subsequent developments have not dampened their optimism. Springtime last year saw a rash of results:

❍ In March 1998, William Langridge and Takeshi Arakawa, molecular biologists at Loma Linda University in Southern California, reported the first evidence of a protective immune response with an edible plant vaccine: mice vaccinated with potatoes expressing a non-toxic fragment (*B subunit* or *CT-B*) of the cholera toxin developed mucosal antibodies to the toxin and when challenged with whole cholera toxin showed a 60% reduction in diarrhoeal fluid accumulation compared with unvaccinated control mice.[20] Extrapolating the data to humans may or may not be justified, but extrapolating uncooked potatoes is definitely not. So the researchers cooked the potatoes and found that 50% of the protein antigen survived intact, which is good news for French fry fans.

❍ In April 1998, an Australian group—Steve Wesselingh, head of infectious diseases at Melbourne's Alfred Hospital, Richard Strugnell of the University of Melbourne, and Ian Dry of the Commonwealth Scientific and Industrial Research Organisation—told a biotechnology conference they had successfully transfected tobacco plants with a measles virus antigen that produced an immune (neutralizing antibody) response in mice.

❍ In May 1998, the first report of a human trial of an edible plant vaccine appeared[21]. The trial, which was conducted by Carol Tacket and her colleagues at the CVD in Maryland in collaboration with Charles Arntzen's group and with backing from the NIAID, showed strong immune responses in 10 of 11 volunteers given 100 g of raw potato engineered to express the B subunit (LT-B) of enterotoxigenic *E. coli* (ETEC), a major cause of travellers' diarrhoea. Commenting in an interview, CVD Director Myron Levine said "6 of the 11 volunteers showed clear mucosal responses and 10 of them had striking increases in neutralizing antibody levels." The only untoward (but unsurprising) effect observed was mild nausea from the uncooked spuds.

Since these developments, a second clinical trial was conducted at the CVD in March of this year with a potato-derived antigen from the capsid, or shell, of the Norwalk virus, another cause of diarrhoea, especially in industrialized countries: 19 of 20 volunteers showed strong responses from cells secreting antibodies (IgA). And in July this year, an oral hepatitis B vaccine produced in transgenic potatoes by the BTI team, in partnership with the British biotechnology company Axis Genetics of Cambridge, entered a Phase I clinical trial: if it passes the full testing and regulatory gauntlet, this plant vaccine will be used to boost immunity to hepatitis B virus in people already vaccinated with the licensed vaccine. The CVD is also planning a "proof-of-principle" clinical trial with a hepatitis B surface antigen expressed in potatoes administered along with a mutant LT-B also expressed in transgenic potatoes. That study, says Dr Levine, is to start early next year and "will really determine whether

Boyce Thompson Institute for Plant Research

Hugh Mason, researcher at the Boyce Thompson Institute for Plant Research, with potato plant

If it passes the full testing and regulatory gauntlet, this plant vaccine will be used to boost immunity to hepatitis B virus in people already vaccinated with the licensed vaccine.

A panoply of promises for edible vaccines

Edible vaccines may not come up to *cordon bleu* standard but if they ever make it into everyday use they could, say aficionados of the technique, offer quite a battery of mouth-watering possibilities. A few morsels to chew on, while waiting:

- Plants can express foreign genes from a broad range of living creatures—viruses, bacteria, parasites, fungi, insects, animals and other plants.
- Transgenic plants can produce large quantities of protein, expressing between 2% and 14% of their total leaf protein in the form of recombinant proteins.
- Plants should be a relatively inexpensive source of vaccine: unlike the microbial fermenters commonly used today by vaccine manufacturers, plant antigens given orally don't have to be separated from their sources of production; nor do they require painstaking, costly purification or rigorous quality control, since plant genes are exposed to far less selective pressure than microbes in fermenters and are therefore much more genetically stable.
- Edible plant vaccines are relatively pure and uncontaminated by animal viruses or other by-products that may be harmful to the vaccine recipient.
- The antigens expressed by plants are as "acceptable," in shape, folding configuration, and so on, and as amenable to immunological processing as the traditional antigens known to produce good protective immunity in humans.
- Judging from animal and early human studies, plant antigens stimulate both local immunity (in gut mucosa) and systemic (generalized) immunity.
- Plant vaccines can be "grown" close to their place of use and may not require the full "cold chain" of refrigerators, ice-packs and so on, needed for many of the traditional paediatric vaccines.
- Edible plant vaccines could make vaccination as palatable an experience as munching a banana and thereby help raise vaccine compliance and coverage rates.

With two Phase I clinical trials completed, a further Phase I trial begun and a Phase II trial planned, the take-home message for edible vaccines is essentially: wait (a bit longer) and see.

Six easy steps to making an edible vaccine

❍ Choose a gene that has already been cloned and that codes for an antigen known to induce protective immunity when administered orally.

❍ Insert the gene into the plasmid of a bacterium, such as *Escherichia coli* (as in step 2 of Box 2 on page 13).

❍ Insert the *E.coli* plasmid into a natural bacterium found in the soil, *Agrobacterium tumefaciens,* that commonly produces tumours in plants.

❍ Choose a plant, like a banana tree, whose fruit most children love to eat. Infect it with *A. tumefaciens* and keep your fingers crossed in the hope that over the next days or weeks this bacterium will insert its own plasmid DNA together with your foreign antigen DNA into the plant's genome.

❍ Take the seedlings of the transfected plants that grow best and produce the greatest quantities of the desired antigen, and plant these seedlings close to where you want to use the edible vaccines.

❍ Harvest your crop of transgenic vegetables or fruit and prepare it—mashed, dried, powdered, etc.—for consumption.

plant vaccines have a future." Meanwhile, Hilary Koprowski's group at the Thomas Jefferson University in Philadelphia, Pennsylvania, is doing animal studies on a vaccine using a rabies virus antigen produced in tomatoes.

To complete the picture and stretch it a bit beyond the edible range, at least two groups are working on using transgenic plants as a source of antibodies for passive immunization.

A British team at Guy's Hospital Dental School in London have tested in four patients a vaccine consisting of monoclonal (i.e. highly specific) antibodies (secretory IgA) to the bacterium *Streptococcus mutans*, the commonest cause of dental caries. The antibodies (or "plantibodies") were produced in a transgenic tobacco plant and prevented *S. mutans* infection in the patients for at least four months.[22]

In the second study, monoclonal antibodies against herpes simplex virus (HSV-2) produced in transgenic soybean and applied to the vaginal mucosa have protected mice against HSV-2 challenge in a study conducted by a U.S. team headed by Kevin Whaley of the Johns Hopkins University in Baltimore, Maryland.[23]

So, with two Phase I clinical trials completed, a further Phase I trial begun and a Phase II trial planned, the take-home message for edible vaccines is essentially: wait (a bit longer) and see. A major gap is the absence of research on what many regard as the holy grail of plant vaccine development: a banana vaccine. The 18- to 24-month lag-time between transfection of a banana tree with foreign DNA and the harvesting of a usable transgenic crop is clearly slowing progress on this potentially rich source of edible vaccines. The BTI researchers, however, say they now have the biotechnology tools to solve this problem.

Boyce Thompson Institute for Plants Research

Cutting-edge research on potato vaccines

Doubts and drawbacks of edible vaccines

Aside from the big question—will they work?—edible vaccines raise a few critical concerns:

❍ The body's immune system does not react to food as it does to an infecting pathogen—fortunately for survival. Will it not become equally tolerant of vaccine antigens presented as food and therefore fail to produce a protective response against future infection? *A human trial on an attenuated* Escherichia coli *toxin grown in potatoes showed a strong immune response to the toxin and no evidence of tolerance.*

❍ Will the protein antigens released by swallowed plant food survive in the hostile gastric environment—biochemically hostile, from natural acidity, and microbiologically hostile, from a plethora of pathogenic organisms infesting much of the infant and child population, particularly in developing countries? *The antigens are protected by the plant's pretty resistant tissues before they are released into the acidic gastric juice, although the degree of protection will depend on the nature of the plant tissue. What's more, some antigens are themselves resistant to acid and enzyme breakdown.*

❍ Will the technology itself survive in the hostile social and political environment that may be created by anti-genetic and anti-vaccine militants, who are likely to join forces over the spectre of children being given vaccines produced by plants genetically modified with the help of bacteria that cause tumours (in plants)? *If edible vaccines are shown to be safe and effective by strict regulatory standards, there is no reason for them to be rejected, whatever the mechanism of their production.*

❍ How easy or difficult will it be to know when a transgenic plant containing a vaccinating antigen is "ripe" for harvesting—not too soon and not too late? *It will depend very much on which plant is used. Current work on alfalfa suggests it can be harvested at any point after the leaf has matured. With a banana, a "promoter" (a DNA signal that turns on the gene expression process) could be chosen that would ensure maximum antigen expression during the ripening stage of the plant.*

❍ How easy or difficult will it be to give the right dose—not too little and certainly not too much—of vaccine in an edible plant? *Clearly, having plant material of uniform quality and consistency will be needed to be sure of having the right dose of vaccine and the right timing of delivery of the vaccine. The approaches used by the food-processing industry to achieve quality and consistency should be applicable to antigen-containing foods.*

❍ How easy or difficult will it be to know whether an individual plant supposed to contain vaccine actually does? How easy, for example, will it be to prevent counterfeit plant vaccines? *Standard assays currently available in medical or industrial research labs around the world could easily detect whether and how much of an antigen is present in a plant sample. And plant biotechnology could create novel (phenotypic) features in plants containing antigens, such as orange-coloured bananas or tomatoes.*

❍ Even transgenic plants grown close to their site of use will probably have to be stored. How readily would a vaccinating antigen retain its potency in a fruit that is going rotten in a storehouse? *Research is in progress on ways of drying edible plant material and processing it to produce, for example, banana baby food puree, dried tomatoes, potato flakes, and so on. Dried leaf material, such as the alfalfa tablets now available in health food stores as protein supplements, may be an option, too.*

Plant biotechnology could create novel features in plants containing antigens, such as orange-coloured bananas or tomatoes.

Sugar glass vaccines (or intimations of immortality)

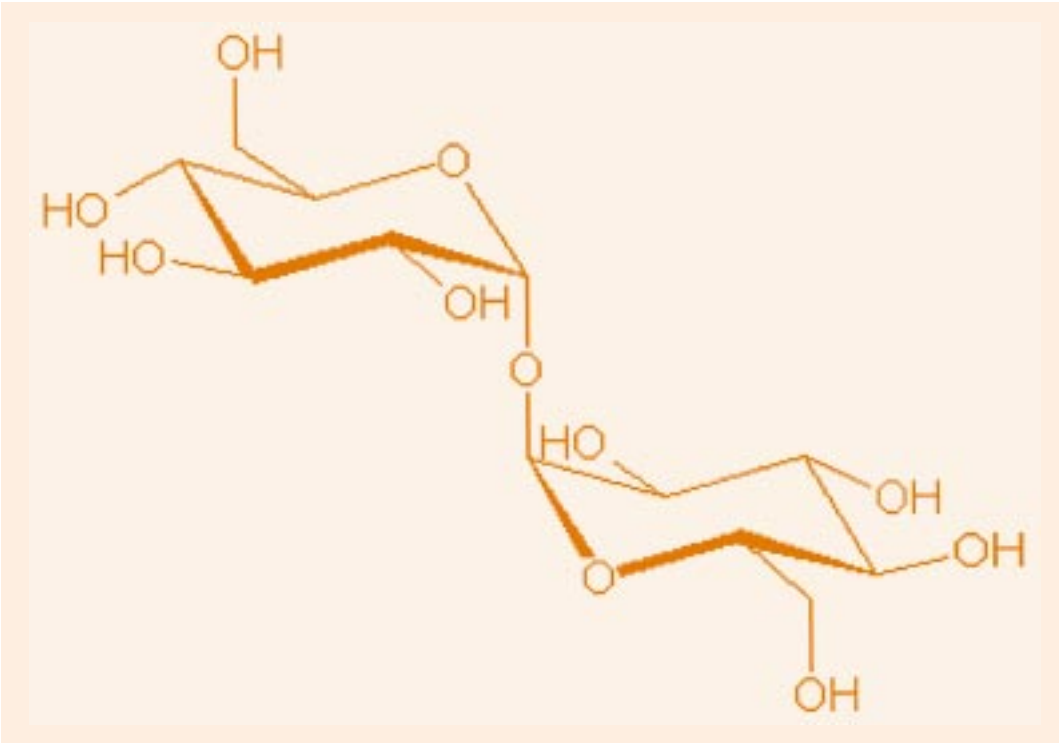
It sounds like magic. Listen to John Lloyd, who four years ago, visited a lab in England, where he was shown a strawberry: "They *said* it was a strawberry, but all I could see was a little crust sitting on a saucer. Then they poured a few drops of water onto it and within seconds a beautiful, shiny red strawberry appeared. It looked like a strawberry, it felt like a strawberry, it smelled like a strawberry and it tasted like a strawberry."

"All I could see was a little crust sitting on a saucer. Then they poured a few drops of water onto it and within seconds a beautiful, shiny red strawberry appeared."

Since then, Mr Lloyd, WHO vaccination logistician and trouble-shooter, has been exploring ways of applying trehalose, the substance responsible for this magic, to vaccines. During their sometimes long journey from manufacturer to end-user the current paediatric vaccines used by national immunization programmes in developing countries are protected from damage by heat and other environmental threats by travelling as freeze-dried (*lyophilized*) powders[24] or through a cold chain of refrigerators and ice-packs. Both methods have their drawbacks. Freeze-drying has two: some vaccines are damaged by freezing and a freeze-dried powder has to be reconstituted as a liquid just before use, with a consequent risk of contamination from unsterile conditions. As for the cold chain, it is expensive—about $2 per fully immunized child or $200 million a year[25]—and can be a logistical nightmare, particularly in mass immunization campaigns.

Trehalose seemed to offer a solution.

Like other double-sugar molecules (*disaccharides*), such as sucrose, maltose, lactose and glucose, trehalose is found in a multitude of biological tissues, from mushrooms to mammals. Many organisms use these sugars' energy-storage properties to keep alive under conditions of extreme drought or cold. Certain desert plants, for example, like *Selaginella* (the "resurrection plant"), use them to survive for months on end in a completely dry state. A worm, *Ditylenchus dipsaci*, has been revived with a few drops of water after 23 years of desiccated dormancy. Bacteria, fungi and other organisms learned this survival trick, termed "anhydrobiosis" (*life without water*), billions of years ago.



Courtesy S. Marcesini

Trehalose— a magic molecule?

Trehalose seems to be the sugar most widely used by living creatures with a natural penchant for suspended animation. It is particularly good at restoring dried biological tissue to its original structural and physiological integrity. And it seemingly does this thanks to its ability, when cooling in a saturated solution, to slide smoothly from a liquid to a viscous and ultimately to a solid glass-like state. The glass—"sugar glass"—immobilises, preserves and protects proteins and other molecules that were in the solution, in much the same way as amber preserves ancient insects. The glass readily dissolves on contact with water and releases its contents, which quickly recover their original form and function. "There was no way I could tell that the strawberry had not come straight from the garden," recalls Mr Lloyd.

Trehalose is used (mainly in Japan) for preserving and sweetening foods and drinks. Its biomedical uses include the preservation of corneal tissue for transplantation and blood for transfusion.

In 1990, Bruce Roser, an Australian scientist working in Cambridge, England, began to devise ways of applying the properties of trehalose to vaccines. Vaccines dried in the presence of trehalose could, he found, be produced in a variety of formulations:

- extruded in the form of sugar glass needles: the needles, each possibly carrying an antigen against a different infectious organism, could be inserted into the skin, where they would instantaneously dissolve on contact with subcutaneous tissue fluids and release their vaccinating molecules at a rate pre-set by adjusting the chemical composition of the trehalose solution; the needles, in other words, *are* the vaccines;
- spun into a floss that could instantly and automatically be reconstituted in saline for injection or crushed to produce needles of microscopic size for insertion into the skin;
- vacuum- or spray-dried to produce a foam or fine powder that could be blown or shot into the skin by a jet injector or sprayed into the nose or mouth by aerosol;
- spray-dried in an ultrasonic nebuliser to produce tiny sugar glass microspheres that encapsulate vaccine antigens (such as DNA plasmids) and could be administered orally;
- suspended in a liquid but non-aqueous, hydrophobic solvent like paraffin oil, ethyl oleate or sesame oil for immediate administration (i.e. without requiring the reconstitution normally needed for powder formulations).

Whatever the trehalose formulation, the vaccines appear to suffer no detectable loss of potency after long periods exposed to heat or freezing. The diphtheria and tetanus (*toxoid*) vaccines, for example, keep their original activity after a year of storage at up to 60°C and a pertussis antigen for up to three months at 37°C and for one month at 60°C. The measles vaccine, which in its usual freeze-dried form, loses over 90% of its potency after two months at room temperature, show no loss of potency as a trehalose powder under the same conditions. Even keeping the influenza and tetanus vaccines below minus 70°C for nine months has no adverse effect on their potency. And if alum, an immune-stimulating adjuvant, is part of a vaccine's formulation, it too emerges intact from the trehalose drying process. A limitation of that process is that it can't be used for complex live whole-virus vaccines, like the oral polio vaccine: somehow trehalose can't get into all the nooks and crannies of these viruses where water molecules can lurk.

Mr Lloyd and other WHO officials who have seen trehalose at work are convinced of its potential. In January they launched a $200,000 two-year "sugar vaccine project." The project, however, faces two major hurdles. One is that trehalose is mired in an intellectual property wrangle between a number of large and several small biotechnology companies on both sides of the Atlantic. Another is that, although several vaccine manufacturers have conducted highly successful experiments with sugar glass vaccines, applications of the technology are of limited interest to vaccine manufacturers, whose most lucrative markets are in parts of the world where extremes of temperatures are not as frequent a threat to vaccines as in developing countries.

The sugar glass immobilises, preserves and protects proteins and other molecules that were in the solution, in much the same way as amber preserves ancient insects.

Cost could also be a constraint. Vaccines using the trehalose technology would, according to one estimate, add approximately 5 U.S. cents a dose to the current 10 U.S. cents price per dose paid by UNICEF for the standard diphtheria-tetanus-pertussis (whole-cell) combination vaccine. A new high-yield process for obtaining trehalose from starch has recently been developed by Japanese researchers, which could help lower the price of the product.

So, for now, the lawyers are at work trying to sort out the legal tangle, with the WHO offering encouragement in the wings while seeking alternative sources of intellectual property.

Whatever the trehalose formulation, the vaccines appear to suffer no detectable loss of potency after long periods exposed to heat or freezing.

Skin patch vaccines

Beauty, as the saying goes, is only skin-deep, but it's often the start of a deeper relationship. So with vaccination. At least that's the view of a group of scientists in Washington, D.C., who look on the superficial layers of the skin as the perfect gateway to many key areas of the immune system.

In a quiet corner of the Walter Reed Army Institute of Research, the group, under the leadership of paediatrician Gregory Glenn, has begun vaccinating human volunteers with skin patches imbued with cholera toxin (CT). CT is a powerful stimulator of the immune system and if it weren't for its toxicity might well have become a standard adjuvant for many candidate vaccines. (Most of the animal studies on CT have used a non-toxic subunit of the toxin.) Yet, neither in its animal work nor in early human trials has the team observed any sign of this toxicity when CT is applied via a skin patch. "Cholera toxin is only toxic under certain conditions," says Dr Glenn. "Given orally, without bicarbonate to neutralize gastric acidity, it can cause diarrhoea. Infused into the lungs in high enough concentrations, it can cause pneumonia. But given via a skin patch, we have safely immunized a number of people without any sign of toxicity."

In animals—mice, guinea-pigs, rabbits, cats and dogs—the Washington researchers have shown that a CT-impregnated skin patch left on the skin for 15 minutes consistently produces a strong, broad immune response. Measurements made two weeks after a single application of the patch show both antibody and cellular (helper and cytotoxic) responses. The antibody response was seen not only in serum samples but also in gut mucosal tissue. The researchers obtained similar results with another bacterial toxin (heat-labile enterotoxin, or LT), from the *E. coli* bacterium that causes travellers' diarrhoea. An LT skin patch, they found, can prevent diarrhoea in mice force-fed with a "challenge" dose of LT.

I'm dreaming of a… skin patch vaccine

U.S. researcher Carl Alving heads the Department of Membrane Biochemistry at the distinguished Walter Reed Army Institute of Research in Washington, D.C. He has published many scientific papers and is known throughout the world for the rigour and seriousness of his work. In his department though, he is also known to be a highly imaginative inventor and, from time to time, a dedicated dreamer.

"There was a meeting of unit chiefs that morning," he recounted to *CVI FORUM*. "I told them of a dream I had dreamed that night. I was splashing around in a swimming pool and I was being immunized with fatty bodies called liposomes through every square inch of my skin. Then I asked my colleagues: Would anybody like to pursue this idea as a collaborative research project? Everybody looked at the ceiling, and I'm sure they thought: Alvy's gone crazy. Except a paediatrician colleague, Greg Glenn, who piped up and said he'd take up the challenge. The early experiments worked beautifully, and that was the start of trans-cutaneous immunization. We've since found we can do it without liposomes."

Drs Alving and Glenn together patented the procedure as its co-inventors.

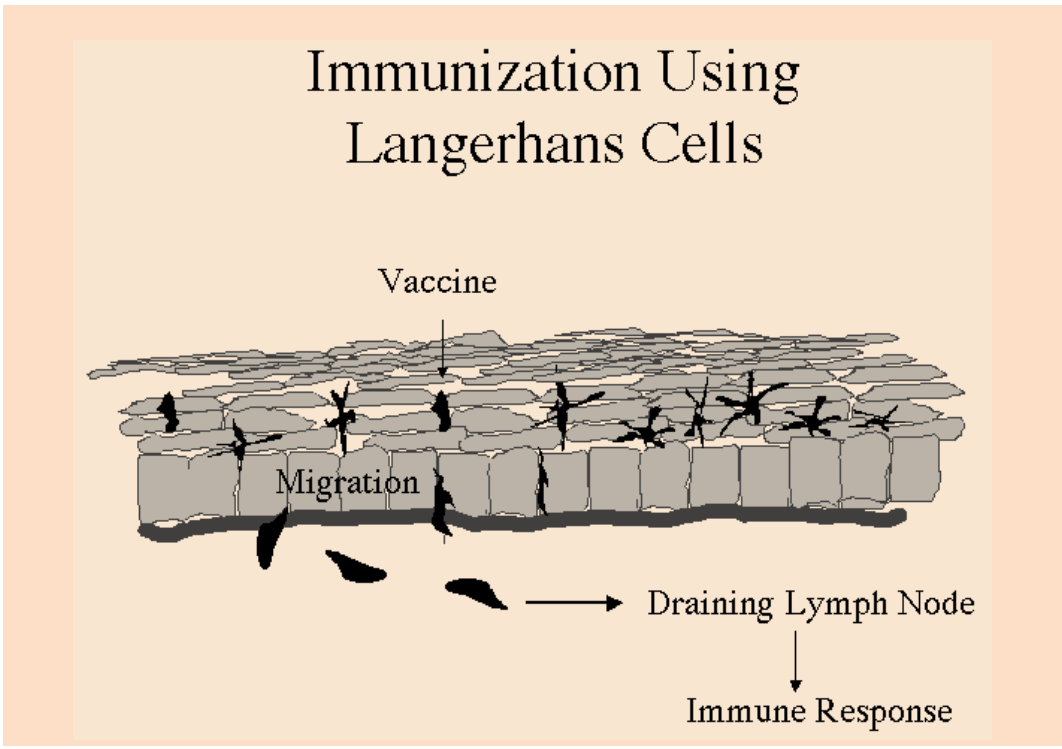
In none of the experimental animals could the researchers find any evidence of toxicity from either CT or LT. Just how these potent toxins lose their fangs when applied to the skin is not clear. One reason, Dr Glenn believes, is that with the skin patch method the toxins don't penetrate far enough into the deeper (intradermal) layers of the skin or into muscle, where they are known to provoke a violent inflammatory reaction.

Dr Glenn's team has also used CT mixed with a vaccinating antigen. To date, they have tested about 30 antigens this way, including those from the causative agents of tetanus, diphtheria, influenza and rabies. Immune responses were observed to each and every antigen tested, without interference from the anti-CT immunity. Wetting the skin before applying the patch, they found, improves the immune response, probably by increasing the permeability of the tough layer of dead cells (*stratum corneum*) making up the outermost skin layer.

For Dr Glenn, the epidermis seems to be a privileged entry point to the immune system. It has an extremely high concentration (up to 1,000 per square millimetre) of immune cells known as Langerhans or dendritic cells, which cover 25% of the total skin surface and whose job it is to deliver foreign antigens to the immune system. "When these professional antigen-presenting cells are activated by the presence of a foreign protein like CT, they carry it to the nearest lymph nodes, triggering a full-scale response involving the entire immune system," Dr Glenn postulates (see diagram). The animal results have been so encouraging that with a few colleagues Dr Glenn has set up a company, Iomai Corporation, to commercialize his "transcutaneous immunization" method.

In a Phase I trial completed last autumn, the team applied a CT or LT skin patch for six hours to the arms of 18 volunteers. Measurements three weeks later showed "a fabulous immune response," Dr Glenn says, similar to that seen in the animal work. "What's more, we saw no problems, no adverse effects, no pathology, either in the skin or elsewhere." Bucked by the results to date, the team, in partnership with researchers from the Johns Hopkins School of Public Health in Baltimore, Maryland, has started a Phase II trial to begin exploring possible *protection* from the skin patch vaccines.

The epidermis seems to be a privileged entry point to the immune system. It has an extremely high concentration of immune cells known as Langerhans or dendritic cells, which cover 25% of the total skin surface.



Courtesy Iomai Corporation

Mechanism of transcutaneous immunization

"If this and subsequent trials pan out as we expect," Dr Glenn says, "we believe our approach, which needs no special skills to apply and which leaves the skin intact, would be ideal for vaccination in rough conditions, like in tropical countries, refugee camps and so on. Several patches could be used, each containing a different antigen, and we could even augment the immune response by putting patches in multiple sites of the body in the vicinity of several draining lymph nodes."

Dr Glenn admits there are hurdles facing his skin patch approach. One is a widespread fear in the vaccine research community of these bacterial toxins—a fear that could translate into a really tough regulatory grilling. But he's plunging on regardless. On Iomai's future agenda are plans to apply the approach to DNA plasmid vaccines, whose immunogenic capabilities (see page 10) could certainly do with a bit of help.

A DNA-based "expression vector" is the centrepiece of another U.S. group working on needle-less transcutaneous immunization—which they call "non-invasive vaccination onto the skin" (NIVS).

Headed by molecular biologist De-chu Tang of the University of Alabama at Birmingham, Alabama, this research too has gone commercial. Vaxin Inc. was set up last year and plans to use the NIVS technology to develop vaccines against a variety of viral and bacterial infections, as well as in certain chronic diseases.

Unlike the Iomai group and its bacterial toxin approach, the Birmingham researchers are using adenovirus to deliver gene vaccines. The vaccine is applied to the skin in the form of a liquid or skin patch. Studies in mice, rabbits and monkeys show immune responses to flu and tetanus antigens. NIVS vaccines are going into Phase I trials in the coming year.

"We believe our approach, which needs no special skills to apply and which leaves the skin intact, would be ideal for vaccination in rough conditions, like in tropical countries, refugee camps and so on."

Meanwhile, back at the bench . . .

Scientists are busy working on a battery of tools and accessories that, although with less media pull, could nevertheless transform the way vaccines are delivered, administered and formulated. The lure is the ideal vaccine or something close—not just effective, but also easy to use and store and transport. Vaccines that can be taken orally or by other mucosal routes have a strong attraction, since they have the potential for combining ease of administration with immunogenic clout. There are currently about 25 mucosally administered vaccine candidates (about a dozen in clinical trials) and at least half of them use one of these novel tools.

UNICEF/Jeremy Hartley

"The variety of widgets and gadgets for delivering antigens is bewildering, to say the least."

Adjuvants

The search for substances able to enhance the immune stimulating capacity of vaccines has produced a real alphabet soup of candidates, from breadcrumbs to tapioca, now numbering close to a hundred. Yet, only one—alum (aluminium phosphate or aluminium hydroxide)—has been universally approved for

use in humans (very recently, another, called MF-59, was licensed in Italy).

Alum, first used in the late 1920s, has a number of shortcomings, among them the fact that it stimulates mainly humoral rather than cellular immunity.

Of the other hopefuls cluttering the pipeline, the adjuvants that work in animals don't always work in tests on people, or are too toxic for such tests, or are stuck in intellectual property disputes.

Still, the search goes on and some vaccine manufacturers, like SmithKline Beecham Biologicals, have set up a whole "adjuvantation" programme.

Certainly, as recent clinical trials of a candidate malaria vaccine have shown, using the right adjuvant can make the difference between a promising result and a flop.

Certain DNA sequences (CpG) and a tree extract (QS-21, from *Quillaja saponiaria*), currently in clinical trials, are thought by some experts to be strong contenders for successful development. Genetically weakened toxin molecules from *Vibrio cholerae* and enterotoxigenic *E. coli* are also favoured by certain research groups.

But the race is still very much on, with a full pack crowding the track.

Live "expression" vectors

A live expression vector is a microbe chosen for its ability to penetrate body tissues and into whose genome a gene has been inserted that codes for a vaccinating antigen of another microbe. Once inside host cells, the vector *expresses* the foreign gene and releases the antigen. At least 59 candidate vaccines, of which nearly 40 in clinical trials, use such live expression vectors, including viruses (adenovirus, fowlpox virus, influenza, polio, rabies, vaccinia) and bacteria (BCG, *Streptococcus gordonii*, lactobacilli, *Salmonella, Shigella*, *V. cholerae*). A sampling of expert views puts BCG, avian viruses, *Salmonella* and *Shigella* high on the list of strong candidates.

Non-living (synthetic) vector systems

This is a clearly a case of human ingenuity let loose. The variety of widgets and gadgets (or "platform technologies," as biotech companies tend to call them) for delivering antigens, each with its fan club, is bewildering, to say the least. Some are conceptually intriguing (bacterial ghosts? hydrophobic feet?). Poking their heads above the crowd, in the view of some experts, are proteosomes (made from the outer membrane proteins of certain meningococcal strains), which have given promising results in clinical trials, and so-called "supramolecular biovectors" (fatty vesicles with sugar molecule cores), also in clinical trials.

* * *

"Not all of these systems will fulfil the hopes of their proponents," says CVI Coordinator Roy Widdus. "But the array of new possibilities they offer is astounding. Those working in vaccination delivery should keep track of developments if opportunities are not to be lost."

As recent clinical trials of a candidate malaria vaccine have shown, using the right adjuvant can make the difference between a promising result and a flop.

Notes

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PICTURE POSTSCRIPT

UNICEF/Cindy Andrew

Setting off into the future, as the CVI bows out (see Editorial, pages 2-4).

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