Prospects for new vaccines

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THE PROSPECTS for the development of new vaccines are often considered to be poor. This is for several reasons. First, the success of vaccination in controlling so many of the scourges of the past, like smallpox, poliomyelitis, yellow fever, diphtheria and tetanus, means that the remaining problems are more intractable from a technical standpoint. Second, many microbial diseases, especially bacterial ones, may be controlled as well or better by other means, by improved hygiene in the hospital as well as in the community and by antibacterial and antiviral agents. Third, the prospects for new vaccines are not improved by various adverse economic factors. The preparation and testing of vaccines is complex and labour intensive. Moreover, it is not profitable and in Western countries the trend is for pharmaceutical companies to abandon vaccine manufacture. For example, in the U.K. there is now only one manufacturer of poliovaccine and in the U.S.A., where the problems are magnified by lawsuits, there is a distinct possibility that there may be no manufacturer of the vaccine, since the only remaining substantial manufacturer produces vaccine in monkey kidney cells and these are becoming less acceptable as well as less available.

Furthermore, the development of new vaccines is becoming more and more difficult and expensive, but yet vaccine development can rarely be protected by patent, so that an innovator cannot be sure of recouping his investment. The clinical evaluation of vaccines is also exceedingly difficult especially if young children have to be vaccinated.

One way in which control of infection can be increased is by improvements in diagnosis. Rapid, accurate laboratory diagnosis of bacterial infections plays a vital part in the control of these diseases. New developments for the rapid identification of pathogenic organisms by immunofluorescence or direct identification of bacterial antigens, for example, polysaccharides of pneumococci, meningococci and *Haemophilus influenzae* have saved valuable time. This approach is of even greater value for the diagnosis of virus diseases. Conventional methods based on serology, or the isolation of viruses in cell cultures impose an inevitable delay in reporting results.

Techniques are now becoming available for the rapid identification of viral antigens in a way that is helpful to the management of illness.

The control of smallpox for example depends crucially on rapid aetiological diagnosis and nowadays this is made most rapidly by electron microscopy which is available in a network of laboratories in the U.K. Electron microscopy is also increasingly used to identify viral pathogens in faeces in cases of diarrhoea; thus the viral causes of non-bacterial gastroenteritis are beginning to be identified. The two most important so far identified are the Norwalk agent and rotaviruses.

Hepatitis A virus has also been identified in faeces by immune electron microscopy and tests for its identification and study in the laboratory are just becoming available. The use of rapid and sensitive methods of detection of hepatitis B antigen, either by reversed passive haemagglutination, or by radioimmunoassay techniques, has improved control of serum hepatitis by defining and refining the areas of risk. There is a real prospect that hepatitis B can be controlled by identifying carriers and avoiding the use of contaminated blood products. Similarly, rigid aseptic precautions enable infected individuals to be safely treated. Different measures including vaccination will be needed in areas where the infection is endemic.

Perhaps the most generally applicable new technique of rapid viral diagnosis is immunofluorescence (Gardner and McQuillin, 1975). This has been applied to the diagnosis of respiratory virus infections including respiratory syncytial, influenza, parainfluenza and herpes viruses. It enables a diagnosis to be made on the day of admission to hospital and has revealed the frequency of cross infection in wards. The technique is also of great importance for the study of immunosuppressed patients who often suffer from undiagnosed virus infections.

The use of these techniques for rapid diagnosis has revealed the importance of viral as well as bacterial infections. They enable management to be improved, to limit the opportunities for cross infection and should enable the new antiviral drugs which are becoming available to be used rationally.

Powerful drugs active against DNA viruses are becoming available, although their value in seriously ill patients has not yet been properly established: there is an urgent need for properly constructed double-blind trials of such drugs because otherwise their value could remain in dispute for several years. Stevens *et al.* (1973) carried out a particularly useful trial of cytarabine in virus infections which shows that despite its value in laboratory infections it is deleterious in immunologically compromised leukaemia patients with herpes zoster. Now even more powerful agents against DNA viruses are available in the laboratory.

Another antiviral agent, interferon, after many years' doubt has been recently reconsidered. This is for three reasons: first, it has been shown by workers at the Common Cold Unit, Salisbury, to be effective prophylactically in man against a rhinovirus (Merigan *et al.*, 1973). In animals, it has been established that interferon can also be used curatively (Finter, 1973). Studies are therefore in progress to establish its value in serious virus infections. Another reason is the possibility that it may be effective in the treatment of some tumours, notably osteogenic sarcoma, and in reducing virus infections in patients with leukaemia. These findings have given the necessary impetus for the production of interferon in considerable quantities for clinical evaluation.

The main purpose of this paper is to discuss the prospects for new vaccines. Table 1 shows some of the new bacterial vaccines at present under study.

TABLE 1. Some new bacterial vaccines

<i>Meningococcus</i>	Polysaccharides types A and B
<i>Pneumococcus</i>	Polysaccharides—many types
<i>Haemophilus influenzae</i>	Polysaccharides type B
<i>Pseudomonas</i>	Surface Antigen 16 serotypes
Cholera toxoid	
<i>Streptococcus mutans</i>	Dental caries vaccines
<i>Gonococcus</i>	
<i>Bacteroides nodosus</i>	Foot rot vaccines for sheep
<i>Escherichia coli</i>	K88 vaccines for pigs

Excluded from consideration are living vaccines against enteric infections which have been under study because the Wellcome laboratories have no direct experience of them. Also excluded are staphylococcal vaccines because although these have been the subject of much work, the prospects for a new vaccine seem as distant as ever.

Although more resources are applied to curative than preventive medicine, the continued prevalence and importance of microbial infections makes a preventive approach attractive. The emergence of bacteria with multiple resistance to antibiotic and chemotherapeutic agents reinforces the case for bacterial vaccines.

An important recent development has been the successful production of vaccines against meningococcal meningitis using the polysaccharides as antigens. The results using this vaccine in the control of meningococcal meningitis and carriage are impressive (Mäkelä *et al.*, 1975) (Table 2). Similar polysaccharide vaccines can be developed for pneumococcal infections but the great number of types is a problem. Similarly, the increasing importance of *Haemophilus influenzae* as a cause of epiglottitis and meningitis makes the development of a vaccine attractive. However, whether this vaccine should be given to young mothers or to older siblings is still a question to be resolved.

Another important infective agent is *Pseudomonas* which is not satisfactorily controlled by existing methods. Workers at the Birmingham Burns Unit (Lowbury and Jones, 1975) have shown that *Pseudomonas* vaccine confers immunity to challenge with virulent organisms in burned mice. They have recently tested an extract of sixteen serotypes of *Pseudomonas* prepared in the Wellcome laboratory and found that antibodies are produced to a single injection in most volunteers (Table 3).

Another application of vaccines is the control of cholera and, indirectly, other gut infections, using the knowledge now available about local immunity

TABLE 2. Effectiveness of meningococcal polysaccharide vaccine

Author	Population	Cases/100,000	
		Vaccines	Controls
Group A vaccine			
Wahdan <i>et al.</i> , 1973	School children Egypt	<1.6 (0)	13 (8)
Erwa <i>et al.</i> , 1973	School children Sudan	<9.1 (07)	7 (65)
Mäkelä <i>et al.</i> , 1973	Service recruits Finland	8.0 (1)	11 (71)
Group C vaccine			
Artenstein <i>et al.</i> , 1970	Service recruits U.S.A.	7 (1)	70 (38)
Gold and Artenstein, 1971	Service recruits U.S.A.	7 (1)	58 (35)

Figures in parentheses are actual numbers of cases.

TABLE 3. Increase in *Pseudomonas* haemagglutinin titre after vaccination (from Jones *et al.*, 1976) expressed as multiple of initial titre

Serotype	Volunteer number					
	10	11	12	13	14	15
1	4	8	2	8	2	8
2	—	64*	8	64	2048	128*
3	4	2	2	2	1	16
4	8*	8*	2	3	128	4
5	4	4	1	4	32	32
6	2	4	1	128	4	8
7	64*	32*	4	256	32	256*
8	16*	16*	4	8	4	16
9	4*	8*	8*	16	8*	32*
10	8*	128*	32	256*	256	256
11	16	2	2	32	64	16
12	16*	512*	32	4	256	32
13	16*	4*	16	4	256	1
14	4	4	2	2	2	4
15	32*	32	8	8	4	16
16	8	16	2	32	4	128

* Pre-vaccination sample lacked detectable antibody.

at mucosal surfaces. This is of two sorts. Cholera and many *Escherichia coli* infections have been established as diseases due to specific bacterial exotoxins. It is in the field of toxic bacterial infections that the greatest successes of vaccination have been achieved, namely diphtheria and tetanus. Hence, it is expected that antitoxic immunity to these intestinal infections should be practicable.

A number of problems occurred: first the method of production of toxoid. In the U.S.A., cholera toxoid after purification by chemical means was found to be unsatisfactory with formalin because of reversion to toxicity. The problem of reversion was solved by using glutaraldehyde, but it is a relatively untried reagent. It could give variable results because it is a more powerful reagent than formalin, and the extent of inactivation will depend upon the precise conditions. This problem has been approached by treating crude material with formalin and then purifying the formol toxoid by adsorption and elution from a specific antibody-containing column (Hughes *et al.*, 1974). Antitoxin has been shown to protect dogs (Pierce and Reynolds, 1974) and the residual problem is to learn how to use vaccine satisfactorily so that antitoxin is effective. Pierce and Gowans (1975) have investigated the best method of producing local antibody in the gut. They examined the appearance of specific antibody containing cells in the thoracic duct and in the lamina propria of the intestines in rats. The maximum numbers of cells were obtained using a combined parenteral and oral dosage schedule; in rats they used a single i.p. dose of toxoid in Freund's complete adjuvant followed by oral vaccine. This is clearly impractical for man, but these findings give definite

clues to the development of an immunization schedule to establish local immunity.

Another vaccine of great interest where these discoveries about local immunity may be important is dental caries. Bowen, Cohen and Colman (1975) have shown that prior inoculation with *Streptococcus mutans* protects monkeys from dental caries when they are challenged with these organisms and fed on jam and sugar sandwiches. A vaccine against dental caries due to *S. mutans* is therefore possible.

Much work has been done at the Wellcome laboratories on vaccines against gonorrhoea. It was thought that the pili of gonococci were very important but this proved not to be so (Turner and Novotny, 1976). This idea was based on the successful development of a vaccine against foot rot caused by *Bacteroides nodosus*, in which pili seemed to be important. Development of a vaccine against gonococci is likely to prove difficult, but the use of chambers developed by Arko (1973) for the study of the disease in laboratory animals and the recognition of differences between organisms grown *in vitro* and *in vivo* (Fig. 1) (Novotny, Short and Walker, 1975) is enabling progress to be made. However, the basis of immunity, or even if it exists, has not yet been established.

Some of the new viral vaccines are shown in Table 4. The ability to diagnose hepatitis B and, in the near future, hepatitis A is enabling the problem of hepatitis requiring vaccination to be defined. It is believed that much of the problem of hepatitis B infection can be solved by the identification of risk factors, carriers, and the use of proper aseptic precautions. These measures have been discussed (Marmion and Tonkin, 1972) in the wake of the serious outbreak of hepatitis B in the renal unit at Edinburgh. A vaccine could be made from 22 nm particles of HB_sAg, and this is being studied in the U.S.A. There are, however, a number of problems. The 22 nm particles of HB_sAg may not contain all the necessary antigens; they contain predominantly antigen a, and there is growing evidence that other antigens, especially antigen e, which is not present in these particles, are important for protection. The material has to be obtained from human donors which may entail risks of transmitting other diseases. The risk is probably small because of the relatively minor hazards of blood transfusion. There is also considerable experience of giving plasma protein fraction (PPF) containing HB_sAg after heating at 60°C for 10 hr without causing jaundice but until the virus can be cultivated no adequate safety test is possible. The only experimental animal available is the chimpanzee. HB_sAg may contain host antigens and the injection of these may be harmful.

Hepatitis A virus is just beginning to be successfully studied by serological methods, but a successful

FIG. 1. (a) Gonococci grown *in vitro*; (b) gonococci grown *in vivo*. (By courtesy of P. Novotny.)

TABLE 4. Some new viral vaccines

Live influenza vaccines
Herpes virus vaccines (e.g. cytomegalovirus)
Hepatitis vaccines A and B
Marek's disease vaccine for chickens

vaccine depends upon the establishment of a successful culture system.

Herpes virus vaccines have proved exceedingly difficult to produce. Living vaccines against a number of veterinary diseases have been successful, for example, infectious laryngotracheitis of chickens, infectious bovine rhinotracheitis and the lymphomatous condition, Marek's disease of chickens. At present, live vaccines against cytomegalovirus in humans are being investigated. They pose serious problems: first, it seems that they may have to be live. Live vaccines are likely to persist, and they manifest themselves later in life if the patient is immunosuppressed either by illness or treatment. Similarly, some herpes viruses cause malignant disease: will the vaccine therefore do so? These are

serious problems and lead to renewed interest in killed vaccines, because if a living vaccine will protect it should be possible to produce immunity with a killed preparation provided the correct antigen is used and the right component of the immune system is stimulated. Powell (1975) has found that glutaraldehyde-treated infected chick kidney cells are as effective as live Marek's disease vaccine for the short term protection of chickens against death from virulent virus. These observations enhance the prospects for preparing a killed herpes virus vaccine.

Influenza vaccines have been prepared for many years, but are not entirely satisfactory. There is now the prospect that new live vaccines may increase our control of the disease. The problems with influenza are to produce an immunity in the respiratory tract with the most recent antigens circulating in the population. Killed vaccines are now capable of being prepared on a large scale and of high purity. However, if epidemic control is to be achieved, a much larger amount of vaccine than is presently used must be prepared to be administered on a mass scale. Much of the pioneer work has been done by Tyrrell

and his colleagues at the Common Cold Unit and by Schild and his colleagues at the World Influenza Centre. The basic technique is to recombine a wild virulent up-to-date antigenic strain with an attenuated strain and to select a progeny virus which contains the genetic material of the wild parent for antigenic structure of the haemagglutinin and neuraminidase with the attenuation of the other parent (McCahon *et al.*, 1973).

In this way, a strain, WRL 105, has been selected (Stealey, McCahon and Freestone, 1975) which has the antigenic characteristics of A/Finland/74 and the growth characters of A/Okuda/57. The attenuated virus chosen is the Okuda strain that was attenuated by more than 270 passages in eggs in Japan. It was used in that country as a live vaccine in about 100,000 people including 9,000 children. It gives rise to recombinants that are attenuated for volunteers and grow well in eggs with reasonable frequency, and appears to be a good strain to act as an attenuated parent. The main problem yet to be solved is to find a reliable marker of attenuation, so that complete reliance on volunteers can be avoided. Another attenuated strain which has been extensively used is the PR8 strain, first isolated before World War II. It has the disadvantage of a long history in the laboratory including passage in mice. Mutagen-induced temperature-sensitive strains, pioneered by Chanock and his colleagues (Murphy *et al.*, 1976), have also received much attention. The approach is attractive because these strains will grow at the temperature of the upper but not the lower respiratory tract. They have some disadvantages. First, the strains have tended to grow poorly in eggs. Second, the strains have shown a tendency to revert, although this has only been found when young children have been vaccinated. Third, the use of a mutagen may have other undetected, undesirable effects.

The WRL 105 strain has been found to be attenuated: thus in trials sponsored by Dr David Freestone, involving nearly a thousand subjects, there have been rather few significant reactions. The vaccine has shown no capacity to spread to contacts and in one trial was effective in preventing the disease (Morris *et al.*, 1975). Most important, it has been established that the vaccine can be given by means of a spray and need not be given as drops. This opens up the possibility of really large scale administration and perhaps interference with the course of an epidemic.

There are many other vaccines that may be developed in future of a quite different sort, and some are shown in Table 1. The ability to make a vaccine against Marek's disease of chickens and the increasing evidence for a role of viruses in human disease make the prospects in this field exciting. Another approach is the use of *Corynebacterium parvum* or B.C.G. to stimulate specific cell-mediated

immunity to tumours. This is successful in mice but whether it will be in man remains to be seen. The prospects for vaccine against pregnancy may seem to be in the realm of science fiction, but at least in the marmoset it has been shown that immunization with the beta subunit of human chorionic gonadotrophin will prevent pregnancy. In principle, at least, such immunization should be reversible by the use of progesterone.

The prospects for new vaccines are thus encouraging, but whether they will, in fact, be achieved is less certain because of ethical, legal and economic problems.

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