ACTIVE IMMUNIZATION AGAINST VIRUS DISEASES IN MAN

A LECTURE*

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It is a well-known fact that the immunity elicited by virus infections is often very solid. Thus second attacks of chicken-pox and mumps are rare. In the case of measles second attacks have been observed, but some of these may be based on incorrect diagnosis and, in the rare authentic cases, many depend on unusual immunity reactions of the host. There are, however, virus infections in which immunity induced by an attack is very poor. We think here particularly of influenza and the common cold. It is worth while pausing to try and analyse the reasons for these differences in the effectiveness of the immunity induced, and to think of the behaviour of viruses in their host cells, before going on to a consideration of active immunization.

During the last decade virology has become a science on its own. An intensive study of certain bacterial, plant, animal and human viruses have brought to light many phenomena fundamental to the main problem we are considering today.

There was a time when, on the basis of the most striking and obvious lesions induced, we regarded viruses as having specific affinities for particular types of cells. Thus we thought of smallpox as a disease in which the virus multiplies selectively in the skin; and of polio virus as entering *via* the nerves of the naso- and oropharynx, from there being transported to the motor cells of the central nervous system, which it damaged or destroyed, thereby eliciting characteristic symptoms. We now know that the polio virus probably multiplies first in the gastro-intestinal tract and enters the blood-stream, only to be taken up in RE or other cells; from there increasing amounts of virus are released into the blood-stream, producing a secondary viraemia, and only then, in a small percentage of the total, does the CNS become affected. The vast majority of individuals acquire the infection at an early age without untoward manifestations and with a lasting immunity. Unfortunately modern hygiene has brought about a changing epidemiological pattern in that infection tends to be delayed beyond infancy and, with the higher age incidence, there is a somewhat larger percentage with recognizable involvement of the CNS.

In the case of smallpox the classical work of Fenner on the corresponding disease in mice (*Ectromelia*) has thrown a great deal of new light. In this disease the primary site of infection is probably in the respiratory tract, but the classical signs and symptoms do not appear until there has been widespread dissemination as a result of primary and secondary viraemic stages. Skin lesions are but the superficial signs of a disease widespread in all the organs.

In measles, too, the eruption follows dissemination of the virus, probably from a primary inhalation infection. Why the rash associated with a disease like measles should have such a characteristic distribution, time of occurrence, etc. is far from clear. It may be that a good deal depends on an allergic reaction, and that it is not the mere presence of the virus which evokes the exanthem.

In sharp contrast to the infections in which viraemic stages are essential in the pathogenesis of the disease, stands influenza. Here the essential infection is limited to the respiratory tract—depending probably on the local spread along the surface of an infection originating in the respiratory tract. No doubt flu virus enters the circulation from time to time during an infection, but the essential disease-manifestations are independent of a viraemic stage.

New facts which have emerged from studies of the relation between viruses and their host cells also have an important bearing on immunity mechanisms. In this virus-host relationship at the cellular level we have learnt a great deal from the bacterial and plant virologist. Although it is dangerous to argue by analogy from bacteria to man there is sufficient evidence to indicate that similar mechanisms may apply.

The action of a fully virulent virus on a fully susceptible cell leads to intracellular multiplication of virus with destruction of the cell and release of a fresh brood of virus. The manifestations of this cell destruction may be immediately obvious in the production of a characteristic disease-picture. It does not, however, need much imagination to realize that there can be a great deal of such virus multiplication and cell destruction without a complex multicellular host like man or animal showing any symptoms.

Even, however, at the cellular level there are differences in virus-host interaction. In bacteriophages it is well-known that virus infections occur which do not do the host any apparent harm. In the so-called lysogenic cultures the bacteriophage infection remains almost entirely latent, and only in the occasional cell does it go on to multiplication and destruction of the host cell. In the intracellular state the 'phage remains quite unaffected by virus-specific immune serum introduced into the environment of the bacteria. An analogous situation occurs in herpes in man. In the human being the primary infection in herpes almost invariably occurs in early childhood—usually as a stomatitis, sometimes as a skin infection complicating, say, eczema. The primary infection is overcome, but many individuals thus infected remain herpes carriers for life. From time to time the appropriate stimulus may bring on an attack of herpes. Thus infection remains latent inside cells of the skin or mucous membrane—in spite of, or perhaps because of, the invariable presence in the

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extracellular fluids of herpes antibodies. The herpes sufferer is immune from reinfection but his immunity is of no value in protecting the cells already permanently infected by the virus.

An even more interesting example of a latent infection is lymphocytic choriomeningitis in mice. In mouse colonies infected with this virus there is very little evidence of the presence of the virus—except the colony's insusceptibility to experimental infection, and the fact that virus can be recovered from apparently normal members of the colony. It is suggested that the L.C.M. infection is transmitted by infected mothers to the offspring during early foetal life—so early that the virus becomes established in the tissues and is not recognized as foreign protein. Antibodies to the virus are therefore not encountered in the mice, in spite of the silent presence of the virus. These mice are resistant to reinfection, not because of an orthodox humoral and cellular immunity, but because the latent virus excludes new and potentially infective virus.

Interesting differences in virus-host relationships are encountered every day by the virologist who attempts the adaptation of viruses to new hosts. Only a few examples need be quoted. Amongst the polio viruses one type (Lansing or Type 2) can be adapted to mice by intracerebral passages. In other words the intracerebral injection of the virus will cause paralysis and death and at post-mortem virus is found to have multiplied in the brain. The same virus injected intraperitoneally into the mice will, except very occasionally, elicit no signs of illness at all, although it almost certainly multiplies in the tissues of the host. Type-1 virus on the other hand, even when injected intracerebrally into mice, does not lead to any manifestations of disease. It can, however, be shown that even in these mice there has been a significant but transitory multiplication of virus.

In some cases virus may on administration to an animal produce manifestations of disease, but no new living virus can be propagated from the lesions by further passage. The virus has apparently succeeded in entering the cells, causing their destruction, but has not completed the cycle of reproduction. In other cases careful adjustment of experimental conditions has resulted in the release of virus which is detectable by *in vitro* methods but is incomplete in the sense that it is not infectious for a new host.

MECHANISM OF IMMUNITY

The attenuation of viruses for the original hosts by adaptation to new hosts probably depends on selection of mutants. It is a method which has been greatly exploited in veterinary medicine for the development of vaccines. It has at present, for reasons which will become obvious, relatively more limited application in human medicine. Blue tongue is a severe epidemic disease in sheep associated with a high mortality. The repeated passage of the virus in eggs increases its pathogenicity for the egg but at the same time reduces it for sheep—so that ultimately large doses of egg virus can be injected into sheep without eliciting symptoms. Attenuated strains have similarly been prepared of horse-sickness, distemper, Newcastle disease and other viruses. In veterinary medicine it is easy enough to test whether such laboratory adapted strains are in fact attenuated for the original host. In human medicine it is not so simple. In poliomyelitis, for instance, we know now that of every hundred or more individuals infected only one will develop the classical paralysis of the disease. Even a fully virulent virus when fed to a hundred individuals may produce no paralytic infection. How many people would have to be exposed to infection before a strain of virus under investigation could be declared harmless? Almost inevitably, indirect methods have to be found to determine whether polio viruses are avirulent for man. Criteria on which the estimation of the harmlessness of strains can be based have been suggested by Koprowski and others. They depend on the response in monkeys and mice receiving inocula by various routes.

The attenuated strains multiply in the hosts into which they are injected but elicit no disease, whilst at the same time antibody production is stimulated. It is probably their multiplication which makes them such effective immunizing antigens. It has often been suggested that immunity in virus infection depends at least in part on the establishment of a permanent latent infection, but no direct evidence verifying this hypothesis has been forthcoming. Quite recently it has been shown by Ackermann that latent infections of cells in tissue culture by polio virus can be brought about by the addition of immune serum to the tissue-culture fluid. A similar mechanism may operate after recovery from natural infections in man and animals, or after the administration of living attenuated strains. It is of course a well-established fact that 'infection' of cells even by partially inactivated virus will exclude subsequently-added fully-virulent virus. The full significance and the extent of the operation of this interference phenomenon in naturally-acquired virus infections is unknown.

Evidence is accumulating also of the production by infected cells of serologically identifiable products other than the virus itself. Whether some or all of these are virus constituents produced in excess or simply by-products of virus synthesis remains to be determined. Their significance in bringing about active immunity is likewise still unknown.

The concept of a permanent latent infection of cells on which immunity may depend is at present almost entirely hypothetical. We should keep in mind the possibility that the latent 'virus' may be in a form not recognizable by the methods which have hitherto been applied, and which like prophage of lysogenic bacteria is intimately associated with cell constituents. Until such 'latent infections' in immune individuals are proven we shall be wise to disregard them in any consideration of practical methods of immunization, but I am bold enough to suggest that we shall hear more about them in the future.

Although specific antibodies detectable by neutralization and *in vitro* tests can be demonstrated in the serum after recovery from practically all virus diseases, there is strong evidence that those diseases which are associated with well-marked viraemia (chicken-pox, smallpox,

mumps, measles) are followed by an effective and lasting immunity. On the other hand, influenza is followed by a poor immunity—probably to be accounted for by 2 important facts: (*a*) The virus spreads along the surface of the respiratory mucosa, where under normal circumstances, antibody concentration is less than in the blood-stream and effective contact with previously-formed antibody does not occur; and (*b*) the virus undergoes frequent significant changes in antigenic structure, so that antibodies against previous infecting viruses are ineffective against new strains.

ARTIFICIAL IMMUNIZATION

We can, I think, lay down certain principles concerning artificial active immunization. Thus we can reasonably anticipate that active immunization will be most effective in those virus diseases in which a viraemic stage is important in the pathogenesis, and in which only a single antigenic type of the virus is known. Furthermore, living attenuated virus is a better immunizing agent than killed virus. A striking example is the effective immunity induced against yellow fever by a single injection of living attenuated virus.

The viruses of blue tongue in sheep and horse-sickness occur in a multiplicity of serological types, so that break-downs of immunity induced with attenuated viruses usually depend on infection with a serological type not included in the multivalent vaccines employed against the diseases.

We could also suggest as a principle the opposite extreme, that active immunity will be induced with greatest difficulty against those diseases in which viraemia is not essential in their pathogenesis, and of which the casual viruses occur in an almost unlimited range of serological types; the striking example here is influenza.

Human medicine will of necessity have to continue to rely on killed vaccines for active immunization against a number of virus diseases which at present constitute unsolved public-health problems. The limiting factors in producing killed vaccines which evoke a significant antibody response are:

1. The ability to produce virus experimentally in high enough concentration. To be an effective antigenic stimulus a certain minimum bulk of antigen has to be administered. It may be that the amount required is significantly reduced by the use of adjuvants of the Freund type, but that the production problem remains significant is well exemplified by the experiences with polio vaccine.
2. The ability to purify the virus or to render it free of harmful antigens.
3. The ability to render the virus non-infective without destroying its antigenicity.

In 2 important virus diseases we are at an interesting stage of the development of prophylactic vaccines—poliomyelitis and rabies. South Africa is particularly concerned, not only because the diseases occur here, but because significant contributions to our knowledge of them have come from South African virologists working at Onderstepoort and the Poliomyelitis Research Foundation.

Poliomyelitis

In *poliomyelitis* it is not the infection of the gastro-intestinal tract but the involvement of the CNS that leads to the disturbing manifestations of the disease. The CNS involvement is almost certainly preceeded by viraemia. The polio virus occurs in any one of only 3 distinct serological types. Protection of the CNS by active immunization should therefore be relatively simple and effective. The development of an effective vaccine against poliomyelitis has therefore been a theoretical possibility for some years. All that was necessary was the production of the 3 viruses in sufficient concentration, free of harmful contaminating proteins (e.g. brain antigens), which on treatment with appropriate physical or chemical methods were rendered non-infectious; or the attenuation of strains in such a way that they became avirulent for man. The former type of vaccine has now been achieved—by the use of monkey kidney tissue cultures, and inactivation by critical concentration of formalin. Not only did the tissue-culture techniques make available a method for the ready cultivation of the polio viruses in large amounts, but the tissue culture virus is free of brain antigens with which virus cultivated in monkey CNS is inevitably contaminated.

The killed-virus vaccine can only be expected to reduce the incidence of paralytic polio—it cannot eradicate poliomyelitis from the community, and gastro-intestinal infections can be expected to continue much as before.

The practical difficulties which face the manufacturer of polio vaccine today are concerned with tests to ensure safety as well as potency, the complete elimination of kidney protein for fear of renal damage or Rh sensitization, and the avoidance of substances like penicillin to which abnormal sensitivity may be induced. The difficulties which face the manufacturers are large, but that they can be overcome as proved by the widespread use of the Salk-type vaccine in many countries today. The unfortunate accidents with this vaccine which occurred in the USA cast some doubts on the safety and efficacy. Further research can still be done to improve the potency and safety of the vaccine, but there can be no doubt that the results of trials undertaken in America in 1954 have been very encouraging and that the South African vaccination programme should be supported.

Several eminent virologists have expressed the view that the poliomyelitis problem will not be adequately solved until live attenuated strains have been developed—which can be fed to young children without risk of producing polio. The difficulty of deciding when a strain is sufficiently attenuated for human use has already been referred to. Furthermore the attenuated vaccine to be effective must establish an intestinal infection, but must not be capable of invading the CNS. Virus in the stools of vaccinated individuals would be infective for contacts—and there is a small risk that man-to-man passage might re-establish its original pathogenicity. In this respect poliomyelitis differs significantly from yellow fever and other arthropod-borne diseases. In these there is no phase during which

virus can be readily transmitted from the vaccinated individual to contacts. When the difficulties mentioned have been overcome vaccination against poliomyelitis may become simple and harmless. Children will then receive the 3 types of virus in attenuated form, by mouth, during early infancy when they still possess a passive immunity transferred from their mothers.

The administration of polio-virus vaccine by the oral route would have the additional advantage that it would follow the natural route of infection, and might thereby stimulate a local immunity. Such local immunity may perhaps be of greater and more general significance than is at present realized. With influenza the importance of local immunity of the respiratory epithelium, at least in experimental animals, has been shown by Fazekas de St. Groth. In mice, vaccination against flu by repeated intraperitoneal injection gives relatively slight protection against inhalation infection. Protection, however, is increased if the mice are given inhalations of homologous or heterologous vaccine after the intraperitoneal immunization. Such 'adjuvant' inhalation results in concentration of antibody in the respiratory epithelium.

Rabies

With rabies the problem is somewhat different. It is a disease which follows the introduction of virus into the tissues—usually by the bite of a rabid animal. Once the disease is established it is invariably fatal. There is, however, a latent period between the time of exposure and involvement of the CNS. During the latent period, the length of which depends on the severity and site of the lacerations, immunization can be undertaken. In South Africa we have for years employed a crude phenolized suspension of the brains of rabbits infected intracerebrally with a 'fixed' strain of virus. The source of most of our human rabies has until recently been wild carnivores in the northern Orange Free State and Western Transvaal. Recently however canine rabies has become a serious problem in the Northern Transvaal. An egg-adapted strain of rabies virus attenuated for animals has been developed, and this is being used effectively in the prophylaxis against rabies in dogs, but human vaccination is still carried out with the relatively unsatisfactory phenolized rabbit brain. The potency of this vaccine has been questioned and its use is attended by the risk of neuroparalytic accidents attributable to auto-immunization against brain antigens which it contains. Limited trials of the egg-adapted virus in human beings have been undertaken without untoward result. In my opinion there is little reason why the living attenuated virus should not shortly be in general use also for human prophylaxis. Unlike the case of polio the establishment of avirulence for man is relatively simple. Vaccination will not be widely administered because its use will be limited to individuals exposed; vaccinated individuals will not become infectious, so that man-to-man passage with reversion to virulence is unlikely. Furthermore there is as yet no evidence that cultivation of the virus in sufficiently high concentration for the preparation of killed vaccines can be achieved in tissues free from brain antigens.

Other Diseases

A great deal of work has been done in recent years on the development of vaccines against a variety of rickettsial and virus diseases. Much of this work is as fundamental as that which has been done on poliomyelitis but it has not had the same 'popular appeal'. The mumps virus for instance can now be cultivated in eggs almost as readily as the viruses causing influenza. The egg-adapted virus is attenuated for monkeys and man. Trials of the efficiency of the attenuated virus in eliciting active immunity against the disease have already been undertaken. An effective mumps vaccine would have a large potential public-health value.

The antibiotics have almost eliminated the dangers of the bacterial and rickettsial diseases. Antibiotics effective against virus infections have not yet been isolated, and for some years to come we shall remain dependent on the virologists and immunologists for protection against virus diseases.

Perhaps the time is not far off when our infants will receive not only a subcutaneous injection against diphtheria and a scratch against smallpox, but also a mouth spray against mumps and a variety of respiratory viruses, as well as a cocktail against poliomyelitis and the flock of intestinal viruses now being isolated.