Preparation of virus vaccines by means of tissue cultures

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Introduction

Within certain limits the body of man and animal is able to offer resistance to the invasion of pathogenic — i.e. disease-producing — organisms such as bacteria and viruses. Depending on the virulence and number of these organisms, however, a percentage of the infected individuals will become diseased, or may even die. In the individuals that survive the disease, antibodies are produced which circulate in the blood serum. These antibodies give the individuals immunity against the disease for a certain length of time, sometimes even for a lifetime. The immunity conferred is specific, that is to say, protection is provided only against a new infection by the same pathogenic organism.

The aim of vaccine inoculation is to give the individual a good start, as it were, by stimulating the body to produce antibodies before infection. Any ill effects which may result through such action should of course be very much less than those of the illness to be prevented.

Vaccine inoculation can be performed with "killed" or living organisms. In the latter case innocuous non-virulent strains of the pathogenic organisms are used.

The method of inoculation with vaccine is largely due to a discovery made by the English physician Jenner in 1796. Jenner found that people could be immunized against smallpox by infecting them with the cowpox virus, which is innocuous to human beings. The name vaccine, from the Latin "vacca" for cow, recalls this discovery.

Vaccine inoculation is a prophylactic measure, applied exclusively with the aim of preventing an illness. Other possibilities are also offered by the use of serum, which again is based on the effect of antibodies. A serum is prepared from the blood of animals that have recovered from a particular infectious disease or which have been inoculated several times with vaccine. Serum therefore, unlike vaccines, contains the appropriate antibodies in a high concentration, and it has an immediate effect. A serum may be administered as a preventive measure, to produce short-lasting immunity, or as a curative measure once the disease has appeared. Vaccines are solely preventive in their effect, but provided they are administered in good time they give more prolonged immunity against infection than serums.

The value of vaccine inoculation may be questioned in view of the outstandingly successful results achieved with modern chemotherapeutics (sulphonamides for example), with antibiotics and with serums. One should bear in mind here that the diseases which have lost their grip on mankind as a result of the use of therapeutic agents are nearly all infectious bacterial diseases and not virus diseases.

A virus is a cell parasite. A virus that invades a cell forces the cell to put its metabolism to the service of the multiplication of the virus, which ultimately destroys the cell. The symptoms of disease observed after a virus infection are attributable not to the large amount of virus produced but to the resultant destruction of large groups of cells, and to the consequent malfunctioning of certain organs affected. But this means that we do not notice a virus disease — and cannot do anything to stop it — until the damage has already made a certain progress. Chemotherapeutics, antibiotics and serums, which have proved so successful in the control of infectious bacterial diseases, are also thrown into the fight against virus diseases but with little success. Viruses are as a rule not susceptible to the common antibiotics and chemotherapeutics and in many cases, because they multiply in the cells and not outside them as is usual with bacteria, viruses are not so readily accessible to these therapeutic agents. Taking this into account, we can understand how valuable vaccines are as a means of preventing virus diseases.

Cultivation of viruses in tissue cultures

In order to prepare virus vaccines, which can be either killed vaccines or living non-virulent vaccines, the viruses have to be cultivated in living matter. A particularly suitable medium for this was found to be incubated (embryonated) chickens' eggs. Many vaccines in use today are made with the aid of chickens' eggs.

A method now rapidly gaining ground is the cultivation of viruses in tissue cultures. The tissues may be taken from any organ of mammals or birds, or of cold-blooded animals, or even insects. They may be tissues from kidneys, testicles, or muscles, etc.

There is a strong reason for making use of tissue cultures, and this is the much greater variety of viruses.
that can be cultivated in this way. We may mention the example of the polio virus, which cannot be cultivated in chickens' eggs but can be cultivated in tissue cultures from a monkey's kidney, a fact to which we owe the possibility of the polio vaccine. Other vaccines which have been prepared by means of tissue cultures are vaccines for measles, foot and mouth disease, canine distemper, infectious canine hepatitis, rinderpest and infectious rhinotracheitis in cattle.

The cell cultures in which viruses are to be cultivated require nutrition. The nutrient medium has to meet very rigorous requirements, particularly in regard to pH, the constituent salts, and amino-acid content. Widely employed nutrient media are those known as "Eagle's solution" and "Morgan's medium" [2]. Given appropriate ingredients of the nutrient medium the cells can be kept alive for a long time, and the metabolism of the cells is kept going in the normal way.

The most frequently used method of tissue culture consists in the formation of primary cell cultures. A particular organ, for example a kidney, preferably young and still growing, is cut into small pieces and treated with a proteolytic enzyme, e.g. trypsin. This treatment has the effect of attacking the structure of the tissue and of detaching cells which, although removed from their structure, remain alive. These cells are transferred to flasks, tubes or other vessels containing a nutrient medium. The conditions are arranged so as to promote the growth and multiplication of the cells, which soon results in the formation of a compact monolayer (one cell thick) at the bottom of the vessel (fig. 1). This is the primary cell culture. After further treatment with trypsin and transfer of the detached cells to a fresh nutrient medium, a secondary culture is obtained, and this procedure can be repeated. If the cells are required to be highly uniform this method of producing a continuous line of cells is the most suitable. Various such cell lines have been described in the literature, the best known being the Hela cell line which, cultivated from a cancerous tumour of a human cervix uteri has now been kept alive for more than ten years [8].

Tissue culture as an aid in diagnosis

Tissue cultures lend themselves particularly well to the demonstration of viruses. The changes produced in a monolayer by infection with a virus can easily be followed under a microscope. The abnormal picture observed some time later, called the cytopathogenic effect and abbreviated to CPE, has a pattern which is usually characteristic of the virus (fig. 2). Most viruses can be differentiated from each other by means of these patterns.

Tissue culture has thus become a valuable aid to diagnosis. The method has in addition led to the discovery of pathogenic viruses which could not have been found by other methods, one example being the virus responsible for infectious rhinotracheitis in cattle. Until about 1956 the true nature of this disease was unknown. The suspicion that it was due to a virus could neither be proved by experiments on animals, nor by cultivation in chickens' eggs. A tissue culture made from the kidney of an unborn calf finally brought the virus to light. Soon after this discovery it was possible to develop a vaccine against this disease and put it on the market.

Once the pathogenic virus is identified the vaccine can either be prepared as killed or as living vaccines. We shall not deal here with the preparation of killed vaccines, but simply mention that a suitable method of cultivation has to be found for the growth of the viruses. While it was formerly necessary to rely on chickens' eggs for this purpose, nowadays there is, as we have seen, a greater choice of tissues.

For the preparation of living non-virulent vaccines the possibility of tissue culture is of very much greater significance. The procedure for finding an innocuous variant of a particular virulent virus is to cultivate various generations of the virus in a host which is foreign to the type of virus concerned. The fact that this usually leads to the desired result may be understood in the following way. In addition to the virulent and rapidly multiplying virus, less virulent variants occur in a strain of viruses, but their existence is entirely overshadowed by the virulent species. This situation may be changed when the virus is transferred to another host:

Fig. 1. Photomicrograph of a tissue culture of a dog's kidney. The culture is a monolayer, i.e. one cell thick. Magnification 100 x.
one or more of the less virulent variants find it easier to adapt themselves to their new environment, and in their turn become virulent to the new host, and become dominant. By repeating this procedure a number of times — that is to say repeatedly transferring a small proportion of the changed virus population to the foreign environment — we are finally left with a population from which the virus which was virulent for the original host has been almost completely removed.

The method described will obviously not succeed in all hosts. When chickens’ eggs were the only medium used for cultivation it was not always possible to develop living non-virulent vaccines. Tissue culture, with its wide variety of hosts, offers much more scope in this respect.

For the development of living non-virulent vaccines not only must the variant produced be non-virulent, it must also give the same or almost the same immunity to the original host as that which results when an individual survives a virulent infection.

It might seem surprising that it should be possible at all to find a virus possessing such a combination of properties. We shall not go into this subject here, but mention merely that this possibility is bound up with the fact that the nucleus of the virus governs the degree of virulence and other genetic characteristics of the virus, and that the protein coat of the virus is involved in the formation of the antibodies (likewise proteinous) in the host and thus determines the immunizing properties.

It will now have become clear that in developing a living non-virulent vaccine it is always necessary to establish what measure of immunization can be achieved.

The immunity conferred by a particular vaccine can be determined by means of a serum neutralization test. In this test, blood is taken from an animal inoculated with a given vaccine and some test tubes are filled with various dilutions of serum prepared from the blood, for example, 1 : 10, 1 : 100 and so on. To each dilution a certain quantity of the appropriate virus is

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added, and a test is made by introducing a little of each dilution on a monolayer to determine the highest dilution at which the CPE is not found. The dilution factor of this dilution is then a measure of the immunity against the added virus.

**Tissue culture versus culture in chickens' eggs**

We now have a broad picture of the principal advantages of tissue culture as compared with cultivation in chickens' eggs.

1) A greater variety of viruses can be cultivated.
2) The measure of immunity obtainable with vaccines can easily be established from the CPE. The CPE is also a simple means of identifying viruses.
3) Vaccines prepared in tissue cultures contain virtually no protein. This is important in as much as precautionary measures are required if vaccines cultivated in chickens' eggs are used on subjects allergic to egg protein.
4) The production of viruses presents relatively few problems. The multiplied viruses finally burst out of the cells of the tissue cultures and enter the nutrient medium. Purification is usually unnecessary, because of the absence of protein. It is only sometimes necessary to remove cell remains by filtering and centrifuging.

Summary. Prophylaxis by means of vaccines continues to be of very great importance today, particularly as a means of preventing virus diseases. This article discusses the method of preparing virus vaccines in tissue cultures, a method which has gained ground in recent years over the method of cultivating viruses in embryonated chickens' eggs. The new method has made it possible to develop various new vaccines against a variety of diseases, including polio and measles, and a number of diseases in dogs and cattle.