Fundamental Principles of Immunization

Presented by The Wellcome Foundation Limited, 1961.
Technical and Scientific Films Ltd (in association with the Film Producer's Guild)

Produced, written and directed by Denis Ward BSc PhD.

Photographed by David A Holmes.
Edited by Harold Holding.
Animation sequences by Guild Animation Ltd.

The drawing of Jenner was made from the bronze by Monteverde in the Wellcome Historical Medical Museum, London.

Colour
Duration: 00:36:57:00

00:00:00:00

<**Opening titles**>

<**Unspecified narrator over drawing of Jenner**>

When, in 1797, Jenner inoculated the arm of James Phipps, a young boy of eight, with the contents of a cowpox bled from a dairy maid, and in the following year published his work on vaccination against smallpox, it became possible for the first time to protect human beings artificially against pathogenic organisms; to immunise them, and a new era in preventive medicine had begun.

<**Narrator over a series of animated illustrations**>

In Jenner's day, when the population of Great Britain was about 10 million, most people hoped to catch smallpox only in its milder form in order to gain protection against an epidemic of the severer kind. Even so, in 1796, one death in every five was due to smallpox. Today, vaccination has completely changed the picture. For example in 1958 with a population of 50 million, there were 6 cases and 1 death. It is now, except for imported cases leading to local epidemics, a comparative rarity, and will remain so, so long as vaccination is sufficiently widely practised. Similar considerations apply to other diseases, but to understand how immunity can be acquired, either naturally or artificially, we must first consider certain basic principles.

If a foreign substance, such as a protein, enters the body of a man or other animal, there is an active response by the production of a specific antidote, which neutralises the toxic effects of the protein as a preliminary to its elimination. Invading foreign substances which act in this way are termed antigens, and the neutralising substances they give rise to, antibodies. This antigen-antibody reaction also operates when pathogenic micro organisms attack the body, since the toxic effects of these are likewise due to protein-like substances either contained in or released by them.

With invading bacteria and viruses, however, the situation is more complicated than this simple picture might convey, since these are living organisms with their own differing characteristics. In virus infections, for instance, such as poliomyelitis in man, and distemper in the dog, the virus itself is the antigen which stimulates the formation of antibody. In some bacterial infections, such as enteric fevers and whooping cough in man, and anthrax and coliform infections in animals, the organisms invade and multiply in the host. The accumulation of bacterial growth is toxic and constitutes the antigen. The protecting antibody that is formed is one that prevents this multiplication.

In another important class, the bacteria may remain localised but release a powerful toxin which diffuses into the bloodstream. In such exotoxic infections, as they are called, which include diphtheria and tetanus, and lamb dysentery and pulpy kidney disease of sheep, it is the toxin which is the important antigen. Since bacteria

belonging to this class are, in some respects, more clear-cut in their behaviour than other bacteria and viruses, it is convenient to discuss them in more detail first.

Let us assume that a completely unprotected, that is to say a susceptible or non-immune person or animal becomes infected with a virulent organism belonging to this class. As we have said, toxin-producing bacteria may remain localised but release a powerful toxin which diffuses into the blood stream to attack a number of vital centres. It is this toxin which is the important antigen, and, as in the case of the protein, the body cells respond actively by the production of antibody. If in the untreated subject the body cells produce enough antibody, or antitoxin as it is called in this case, rapidly enough to neutralise the toxin before irreversible damage has occurred, recovery will follow. Antitoxin will then predominate.

As a result of a clinical attack of a bacterial disease, the status of the subject is in many cases changed from non-immune to immune. Though the concentration of antibody in the blood may drop after the lapse of time when it is no longer needed, what has happened is that the body has become trained, as it were, in the mechanism of its production, and a pattern for the rapid reformation of fresh antibody persists, represented here by these blue-ringed circles.

00:05:26:00

Sometimes, even repeated sub-clinical infection will produce a similar result, and should a further attack be experienced, the body will almost always produce antibody rapidly and in such quantity that clinical symptoms are unlikely to appear. Immunity acquired in this way, by reaction of the body itself, is called active immunity, and when naturally conferred by an attack of some, but not all infectious diseases, is fairly solid. A more lasting immunity than that following an attack can, as a general rule, be induced by artificially stimulating the production of antibody such as antitoxin.

Into a non-immune person or animal we can inject what is called a toxoid, which is, in fact, toxin rendered non-toxic, and therefore harmless, by chemical treatment (for

example, with formalin), but which is sufficiently like toxin to retain the capacity to stimulate the body to produce antitoxin.

A single injection of toxoid or one of its derivatives which may sometimes be used, is seldom sufficient to produce measurable immunity, but will educate the body to respond quickly and efficiently to a subsequent stimulus. The antitoxin produced appears in the blood serum and is measurable. In this way it can be established that a single injection of, for example, one of the potent diphtheria prophylactics, results, at best, in a very low level of antitoxin, which even at its maximum after 4-6 weeks, is well below that required to confer protection. Such a minimal, or primary response, has resulted from what is called the primary stimulus. If, however, a secondary stimulus is given, at a suitable interval after the primary response, a secondary response ensues in which a comparatively high concentration of antitoxin is produced in the blood serum. The concentration reaching its maximum about 10 days after the injection, and thereafter falling off relatively slowly.

A high degree of immunity can be induced in this way, though with time it gradually falls. By reinforcing doses given from time to time, however, it is capable of rapid restoration. The phenomenon of primary and secondary stimulation was discovered by Glenny and Sudmersen in the Wellcome Research Laboratories in 1921. And today it forms the basis of all accepted methods of immunisation with toxins or their toxoid-derivatives. Again, as occurs in many cases of recovery from a natural attack, the inoculated subject is now actively immune. Again, the pattern for the rapid reformation of antitoxin is present, but as we have seen, the basic immunity which enables this to happen, takes time to develop.

But there are many occasions, for example, when the non-immune person or animal is exposed to heavy infection, when there is no time to allow the comparatively slow processes of active immunisation. In such cases, antitoxin is urgently required, and resort must be had to direct injection of blood serum containing antitoxin in enough quantity to neutralise the toxin as it develops. To protect, such antitoxin must be injected promptly, and is in fact best used at the earliest possible moment after exposure to infection. Serum containing antitoxin for use in this way is also called

antiserum and is usually produced in a horse, a particularly suitable animal for this purpose.

The protection afforded is, however, short-lived, because the serum, not being produced by the subject's own body, and thus being a foreign protein, is eliminated comparatively rapidly. Temporary immunity conferred in this way, by injecting antibodies, is called passive immunity and can be contrasted with the active immunity produced by inoculation with antigens such as toxoid. Passive immunisation is readymade help in combating disease, in which the body plays only a passive role; it is rapid in onset, but of short duration. Active immunisation, on the other hand, is a means of training the body to protect itself by its own activity; it takes an appreciable time to develop but is long-lasting. This difference between active and passive immunity is fundamental.

00:10:23:18

It is important to remember, however, that immunity, either active or passive, to one disease can boast no protection against other completely unrelated diseases. There are then these two basic types of immunity – active and passive.

To recapitulate: active immunity may be acquired naturally by recovery from an attack of disease or by sub-clinical infection; preferably active immunity may be induced artificially by inoculation with an antigen. In all active immunity, the subject's body itself is trained to produce antibody, and immunity is slow to develop, lasting in character and when it falls, generally capable of rapid restoration. Passive immunity may be inferred by inoculation with antibody produced outside the patient and in this case immunity develops rapidly, but is transient in character.

Although so far we have only discussed exotoxic infections, these principles nevertheless are capable of more general extension, though there are, as might be expected, variations and exceptions from disease to disease as well as between the different classes of disease.

Before drawing attention to some of the variations, however, we must first consider certain practical consequences which arise out of the basic principles. As we have said, antiserum for passive immunisation is usually produced in a horse.

<**Narrator over shot of horse and laboratory work to produce antiserum**>

After a period of isolation and other preliminaries, the animal undergoes an intensive course of active immunisation by repeated inoculations with antigen. In this way, it is brought to what is termed, a hyper-immune state, in which the blood serum is rich in antibody. Periodic bleedings from the jugular vein are then taken. This procedure can be adopted for the preparation of antisera for passive immunisation against various bacterial infections.

After bleeding, the antiserum is refined and concentrated. As we have already said, horse serum when injected into a different species constitutes a foreign protein which is eliminated more or less rapidly; and it is for this and other reasons, including the risk of undesirable serum reactions, that concentration in plants like this, to bring the radio of wanted antibody to unwanted serum protein to the highest possible level, is important.

In the case of antitoxic sera, such as that of diphtheria for example, the antitoxin-bearing fraction can, as we see here, be separated by a process involving pepsin treatment at a controlled pH. This is followed by heat denaturation and precipitation of the purified antitoxin in the filtrate with ammonium sulphate. The precipitated cake contains the desired fraction.

The ammonium sulphate is subsequently removed from the precipitate by dialysis in cellophane sacks; to describe the process as simply as this is, of course, to over-simplify. But the details are less important than the reasons for concentration. To separate pure antibody, however, remains a practical impossibility. However carefully they are concentrated, antisera prepared from the horse always contain some unwanted protein in addition to the antibody protein and both are horse serum proteins.

Valuable and important as such products are, therefore, here tetanus antitoxin is being automatically filled into ampoules under completely sterile conditions; considerations of this kind suggest that if we wish to take greatest advantage of passive immunisation to ensure that the antibodies remain as long as possible in the body of the subject, we should make use, not of heterologous antisera from the horse, but of potent homologous antisera from the species we wish to protect. Unfortunately, this too is not usually practicable except in the case of some virus diseases.

<**Narrator over young male patient in bed donating blood, then a man donating blood**>

In measles, for instance, blood serum taken from a preferably young patient, a week or so after the temperature has returned to normal after a natural attack, has a useful value as a passive immunising agent. Such antiserum is called convalescent serum but is obviously not widely available. But with the increase in the blood donor service and since people are exposed to many different bacteria and viruses, large pools of blood plasma are now available which normally contain a variety of protective antibodies. These are located mainly in the gamma fraction of the globulin, which can be separated.

<**Narrator over shot of GP giving vaccination to a baby, then a dog**>

Gamma globulin from pooled adult plasma has been recommended for the passive immunisation of susceptible contacts against some virus diseases, such as measles, rubella and poliomyelitis. But it’s been stated to be ineffective against others such as mumps or chickenpox. In some cases, gamma globulin containing more specific antibodies, such as that prepared from the blood of persons recently vaccinated against smallpox is of value. Or, for example, a dog, the owner of which failed to have it actively immunised, may be protected for a period against canine distemper by passive immunisation with antiserum prepared from the blood of another dog, actively immunised against the disease.

In all such cases, since the serum is homologous, the body is less prone to reject it hurriedly and the protection conferred is somewhat longer lasting than is possible with heterologous antisera.

00:17:09:00

<**Narrator over animated illustrations**>

We thus have a further important principle. Passive immunity can be conferred either by use of heterologous antisera, prepared usually in the horse, or by use of homologous antisera prepared in the same species. With heterologous antisera the protection conferred is relatively brief since they contain foreign protein which is rapidly eliminated. With homologous antisera on the other hand, the protection is somewhat longer lasting. In both cases, however, the effect is only temporary since in any form of passive immunisation, the body itself is in no way trained to produce its own antibody. The protection conferred, therefore, is markedly inferior to that induced by active immunisation.

But this is not the whole story. Suppose a woman is actively immunised, and that the level of antibody in her blood serum is high. Suppose now she gives birth to a child. The blood serum of the child will also be rich in antibody, which, in the case of man, apes and rodents, has been transferred from the mother to the offspring mainly by means of the permeable placenta. This transferred immunity is, however, passive; again, the antibody has not been produced by the child itself.

A similar transference of antibody occurs in certain other mammals. But in the case of ungulates such as cattle and sheep, this takes place not by the placenta, which is impermeable in these species, but via the colostrum or first milk which is ingested into the gut and from which antibody is absorbed into the blood stream of the offspring. We thus have yet another important principle, that active immunity in a mother can be transferred as passive immunity to the offspring. But it must be remembered that though transferred by the most homologous means possible, such

immunity in the newborn child or animal is still passive in character and is, therefore, of limited duration. From this principle of transferred immunity, some important consequences follow.

<**Narrator over shots of sheep and lambs**>

For example, in hill sheep farming, lamb dysentery at one time caused losses of up to 40% of the lambs. This disease only attacks within the first few days after birth and, plainly, sheer inaccessibility may prevent the passive immunisation by heterologous antiserum of each lamb immediately it is born. While even impracticable, active immunisation of the lambs is out of the question since there is not time for the immunity to build up before exposure to infection.

But by active immunisation of the ewes, so enabling the lambs to be passively protected following the first feed of colostrum, the mortality rate from lamb dysentery can now be reduced to negligible proportions.

<**Narrator over shots of a dog and puppies, then a woman and baby outdoors, then visiting a health centre**>

But there is another side to the picture. In protecting dogs against canine distemper, for instance, where active immunisation is effected by use of an attenuated living virus as antigen, the presence of transferred maternal immunity may prevent multiplication of the virus if we attempt to immunise puppies at too young an age.

For this reason, active immunisation must be delayed until the transferred passive immunity has faded. Similar considerations apply to babies born of mothers actively immunised against poliomyelitis in their own interest, during, or before their pregnancy. Here too, transferred immunity would interfere if an attempt were made to immunise the child actively too early.

But active immunisation remains, nevertheless, by far the better way to protect both human beings and animals against infection and should be preferred wherever possible.

As we have indicated, pathogenic organisms may be divided into three broad classes: viruses, bacteria which invade and multiply in the host and do not produce a soluble toxin, and toxin-producing bacteria which last class we have already discussed in some detail, and to which diphtheria belongs.

<**Narrator over animated illustrations**>

As we have seen, though toxin-producing bacteria may remain localised, they release a powerful toxin, which, in a natural attack is in fact the important antigen. This, however, is usually far too toxic for use as an antigen in active immunisation. Instead of this, therefore, the harmless toxoid, or toxoid derivative, is employed.

<**Narrator over shots of laboratory preparation of toxoid**>

To prepare the toxoid, of course, the toxin itself must first be produced by culture of selected strains of the bacteria. *Diphtheria bacilli*, for instance, may be grown under artificial conditions in a suitable broth to produce a fluid rich in toxin.

After filtration, a suitable concentration of formaldehyde is added to the crude toxin, after which it is transferred to glass bottles for incubation. Incubation takes 2 or 3 weeks and as a result of this treatment, the non-toxic toxoid is formed, which produces no unfavourable symptoms when injected into guinea pigs in large doses. It is, however, still antigenic in that it still possesses the property of stimulating the formation of antibody.

The toxoid is purified, in this case the desired fraction is precipitated by ammonium sulphate and is separated by filtration. Treatment of the respective toxins with formaldehyde is also used for the preparation of other toxoids, including those for active immunisation against lamb dysentery, black leg, braxy and pulpy kidney

disease in animals; and tetanus and botulism in man. Such toxoids are often referred to as formal toxoids. Where desirable, these too may be submitted to a process of purification.

In some cases, such as those of diphtheria, braxy, pulpy kidney disease and others, the toxoid may be precipitated or absorbed with a preparation of alum before use. This gives a relatively insoluble product which is more slowly absorbed and excreted than formal toxoid itself. And which, because of this, possesses enhanced antigenic efficiency. The original discovery of this use of alum was made in these laboratories.

As with all other biological products, toxoids and their derivatives are, of course, filled into bottles and ampoules under completely sterile conditions. We have referred to products in this class as toxoids, but this term is now less used than formerly and is usually applied only to those toxoids intended for active immunisation of human beings. For all products used to induce active immunity, including toxoids for use against animal infections, and increasingly those against human infections also, the more general term, vaccine, is being preferred.

00:25:20:09

<**Narrator over animated illustration**>

Vaccines can also be prepared for active immunisation against diseases due to pathogenic bacteria which do not produce a soluble toxin. We are, therefore, unable to obtain a toxoid and recourse must be had for the antigen to the bacteria themselves.

<**Narrator over shots of laboratory work to prepare whooping cough vaccine**>

In the case of whooping cough, for example, *Bordetella pertussis* may be grown in a special culture medium, designed to reduce the amount of unwanted foreign protein in the finished vaccine. The medium, contained in 2 ½ litre flasks, is inoculated with a suspension of the bacillus.

After incubation for a suitable period, the bacteria are killed with formaldehyde. Vaccines exist in this class essentially of killed bacteria, since with the exception of tuberculosis, where the well-known BCG vaccine can be used, it is not possible to employ living attenuated strains in the human field; although there are a few such veterinary vaccines.

Sampling to test for the purity of the strain, centrifuging to separate the killed culture and addition of an antibacterial substance are followed by standardisation of the adjustment of the opacity of the suspension to a standard value.

Antigenic activity is assessed by making a challenging intracerebral injection of living culture into anaesthetised mice which have been previously inoculated with the vaccine under test. Active immunisation is possible with varying degrees of success against most diseases caused by pathogenic bacteria which do not produce a soluble toxin: such as the enteric fever group, cholera and plague in man and pasteurellosis and swine erysipelas in animals. Here, swine erysipelas vaccine is under inspection before packing. Largely because killed bacteria are used, however, more frequent re-inoculation may be required than in the case of infections due to toxin-producing bacteria.

Though passive immunisation with antisera is of some value in the treatment of certain veterinary diseases in this class, such as swine erysipelas, coliform infections and pasteurellosis, passive immunisation in man has been largely abandoned in favour of chemotherapy for the treatment of infections in this group.

<**Narrator over animated illustrations**>

In the third, and important class of viral infections, again the whole organ is the antigen. Again, there is no soluble toxin and again we must have recourse to the organisms themselves. But here it is more often possible to use not inactivated killed organisms but attenuated living strains, which, although harmless to the host,

nevertheless can be used to induce fairly solid active immunity. This is an important principle.

If a virulent organism from one species is introduced into a second species, represented here by this triangular symbol, the organism may develop, but in so doing may change in character or become attenuated. But because the attenuated organism is so closely related to the virulent type from which it is derived, it may still possess high antigenic activity against infection by the virulent type. So that when reintroduced back into the original species, it affords protection, though it is no longer virulent for that species.

In a similar way, the vaccinia virus provides protection against the closely allied smallpox virus. Strains attenuated by various methods are used for the preparation of vaccines for use against a number of both human and veterinary virus diseases, including yellow fever and poliomyelitis; [..]

<**Narrator over shots of laboratory work using chick embryos**>

[…] and canine distemper (hard pad) and rinderpest.

In the case of canine distemper, the species used for the attenuation and growth of the virus is the chick embryo. The living virus is inoculated into the region of the embryo of hens' eggs incubated for 8 days. A similar procedure is adopted in the case of yellow fever. After a further period of 6 days incubation, the virus concentration reaches its highest level in the embryo which is then removed, prior to homogenising and separation of the vaccine and filling into ampoules.

Because such vaccines are unstable in solution, they are finally freeze-dried in this apparatus. The dried vaccines retain their potency for some months if stored at 5˚C. They contain the living virus in a state of suspended animation.

The potency is determined by reconstituting the vaccine and estimating the virus concentration by inoculation onto the chorioallantoic membrane of embryonated

eggs. Macroscopic lesions develop after 5-6 days – the active immunity induced by the use of attenuated living virus vaccines is usually high. In some cases, however, vaccines are prepared containing not living but killed viruses. Here, for example, poliomyelitis strains grown on monkey kidney tissues, and inactivated by formaldehyde, are being blended to produce a vaccine of the Salk type. There are, of course, living attenuated vaccines available as well.

Samples taken are tested by inoculation of monkeys. The protection afforded by most killed virus vaccines is, as might be expected, somewhat lower than that given by living attenuated strains. Moreover, antisera for passive immunisation against virus diseases are generally only effective if homologous.

00:32:27:00

<**Narrator over various laboratory shots depicting vaccine production**>

In this short account, we have only been able to touch upon but a few of the principles which lie behind the various methods available today for conferring immunity against both human and veterinary infections. And there is much that has been left unsaid. We have, for example, said nothing about the use of combined prophylactics such as diphtheria-tetanus-pertussis vaccine or braxy-blackleg-pulpy kidney vaccine. Or about the possible adjuvant effect which one antigen may exert on another. We have said nothing about the possible role interference in virus diseases. The subject is vast, and its complexity as great as the multiplicity and complexity of disease itself. Nevertheless, certain fundamental principles emerge.

We note that we can distinguish between 2 basic forms of immunity – active, in which the body itself is trained to produce its own action; and passive, in which the body plays no direct part but in which aid is brought in from outside. We note the important principle, that active immunity is, in general, slow to build up, lasting in character, and when it falls, capable of rapid restoration. Whilst passive immunity develops rapidly but is transient in effect. And we further note, that active immunity in a mother may be transferred as temporary passive immunity to newly-born offspring.

Against diseases due to toxin-producing bacteria, like diphtheria and tetanus in man, and lamb dysentery and pulpy kidney disease in animals, we note that we can induce a high degree of active immunity by use of toxoids or their derivatives, and confer useful passive protection by use of antitoxic sera.

Against diseases caused by pathogenic bacteria which do not produce a toxin from which we can prepare a toxoid, such as whooping cough and enteric fever in man, and swine erysipelas and pasteurellosis in animals, we not that we can often induce useful immunity by use of killed bacterial vaccines, though, with the exception of some animal diseases, we also note that in this class chemotherapy has now largely superseded passive immunisation in treatment.

Against virus infections such as yellow fever in man and canine distemper, we note that we could induce a high degree of active immunity by use of living attenuated virus vaccines; and against infections such as poliomyelitis, a useful degree of protection by use of killed inactivated virus vaccines. We note, however, that passive immunity in this class is usually obtained by the use of certain homologous antisera produced from the same species.

These then are the fundamental principles which today form the basis of all modern immunising procedures; both human and veterinary. Principles and procedures which have enabled major advances to be made in the control of disease and which, together with improved methods of hygiene, have reduced many one-time major scourges to negligible proportions.

<**Narrator over shots of prophylactic products, then the original drawing of Jenner**>

The range of prophylactics is great and through continued research is ever becoming even greater. We have indeed come a long way from Jenner who, over 150 years ago, laid the foundation of this important and ever-growing science of immunology.

<**End credits**>