than for the information it conveys, for not infrequently union is firm to clinical tests some weeks before it is confirmed radiologically. This third *x*-ray depicts the anatomical result, but nothing less than a film will disclose the restoration of function, and even this does not record the various aches and pains which may impair a perfect result.

It is strange that public opinion on the treatment of fractures is that anything short of perfection indicates incompetence or negligence, and yet a much greater margin of error is given to us in the treatment of almost any disease. But most of the latter tend to get well, and in some fractures, particularly if occurring after middle life, we cannot anticipate more than a fair result.

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COMBINED USE OF LIVING VIRUS AND IMMUNE SERUM FOR IMMUNIZATION AGAINST VIRUS INFECTIONS

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None of the methods at present employed in immunization against virus infections is completely satisfactory. Since the time of Jenner it has been recognized that the use of a virus attenuated by passage through another animal species can confer a high degree of immunity. Nevertheless, the employment of such an attenuated virus may not be entirely free from danger, as is shown by the occasional occurrence of an encephalitis after inoculation with vaccinia virus. Whether due to the vaccinia itself or to some other associated virus, the encephalitis appears to be initiated by the lesions produced as a result of the local inoculation of the vaccinia virus, and in order to minimize these lesions a single inoculation, with a minimum of trauma in place of the customary four insertions, has been officially recommended (Ministry of Health, Committee on Vaccination, 1928).

Various other methods of immunization have been tested, in particular those involving the use of virus attenuated by chemical means. Although these vaccines have given good results in dog distemper (Laidlaw and Dunkin, 1926 and 1928), rinderpest (Curasson and Delpy, 1926; Kelsner, 1927), fowl pest (Todd, 1928), and yellow fever (Hindle, 1929), there are certain technical difficulties in their preparation. One of the chief of these is the absence of any reliable method of standardization, so that vaccines of varying degrees of potency are obtained, and it is impossible to titrate them even approximately, except by making numerous animal experiments. Moreover, in dog distemper it has been found advisable to reinforce the immunity following vaccination by a subsequent injection of living virus, a procedure fraught with many technical difficulties, and only possible in the case of animals, since any failure in the potency of the vaccine must certainly result in a generalized infection.

The nature of these vaccines is still under discussion, but there is strong evidence in support of the view that many of them must contain living virus. Up to the

present, all attempts to prove the existence of live virus in yellow fever vaccines have given negative results, nor does the blood of vaccinated monkeys become infective to mosquitos. However, the possibility of such an eventuality cannot be entirely ignored.

Immune or hyperimmune serum has been advocated in certain virus diseases, and in particular has been employed for protection against yellow fever. Although this method possesses the advantage of producing an immediate effect, in contrast with vaccines which require at least several days before conferring any protection on the subject, it has the great disadvantage of producing only a temporary immunity instead of the more durable protection conferred by vaccines. The results obtained by the use of immune serum in yellow fever are discussed below; they indicate that, as a general rule, this passive immunity rarely persists for more than a few weeks; similar results have been obtained in rinderpest and hog cholera. Yet another method of protection is obtained by a combination of immune or hyperimmune serum and virus, which has been used for immunization against both rinderpest (Kolle and Turner, 1898), and hog cholera (Dorset, Bolton, and McBryde, 1904). In the case of rinderpest the animals were inoculated on one side of the rump with immune serum, and on the other side with virus. Some individuals reacted with a fever, and their blood became infective, and about 1 per cent. died of the disease; the application, therefore, of this method in rinderpest is restricted to heavily infected localities. A further difficulty has been the risk of infecting with piroplasmiasis or trypanosomiasis. In the case of hog cholera the use of immune serum produces a passive immunity of about three weeks' duration, but if combined with virus confers an active immunity which lasts for at least six months, and probably longer. The inoculated animals may show virus in the blood, and numerous deaths have been recorded, especially in the earlier experiments. Probably many of these earlier fatalities may be attributed to faulty technique, for later observers—for example, Huttyra and Köves, 1916—tested the method in Hungary and immunized 20,000 hogs, with very slight losses, or no losses at all.

Laidlaw and Dunkin (1931) have recently found that while mixtures of virus and immune serum injected together do not establish immunity, the injection of serum and virus simultaneously side by side does confer durable immunity. So far as we are aware this method has not hitherto been applied in any human infection. Unquestionably, very great care would have to be exercised before it could be generally employed in such a fatal disease as yellow fever, but by the use of immune or hyperimmune serum of proved value there would seem to be very little likelihood of any untoward results. In more benign infections, such as vaccinia, a combination of immune serum and virus, if equally efficacious in producing immunity, would have many advantages over ordinary vaccination, since, apart from suppressing any local reactions, the occurrence of undesirable after-effects would be greatly lessened.

Consequently, we have made experiments to determine whether a satisfactory degree of immunization might be obtained by the use of this method in two very dissimilar human infections—namely, vaccinia and yellow fever. In these experiments active virus was preferred to attenuated virus or vaccines, as, when combined with immune serum, no local or general reaction was observed, and it seemed likely that a fully active virus would produce a more lasting effect than an attenuated one. This method combines the advantages of giving an immediate protection, as a result of the immune serum, and a lasting effect, probably as a result of certain changes in the tissues of the host produced by the introduction of the living virus.

IMMUNIZATION AGAINST VACCINIA BY HYPERIMMUNE SERUM AND VACCINIA VIRUS

The object of these experiments was to determine whether it is possible, by a careful adjustment of the amount of living vaccinia virus and immune serum injected, to produce immunity in the rabbit in the absence of any general reaction, or of any local reaction at the site of inoculation. There is already considerable evidence to show that such a result is possible. Using a strain of neurovaccinia, Andrewes (1929) found that immune serum given intravenously to rabbits, at the same time as large doses of virus inoculated intradermally, prevented generalization of the vaccinal lesions, although development of the local lesions was practically unaffected. Serum given subcutaneously or intradermally could not be relied on to prevent generalization. Of the rabbits in which generalization was prevented by immune serum, 55 per cent. were subsequently found to be immune.

While the experiments recorded in this paper were in progress, Rhoads (1931) showed that vaccinia virus (neurovaccine) and specific immune serum, mixed in such proportions as to produce no lesions when inoculated intradermally, induced immunity when instilled into the nasal cavities of rabbits. The mixture was also effective when inoculated subcutaneously. Immunity was brought about with a minimum of systemic reaction and no local lesion, though when the mixtures were incompletely neutralized generalized vaccinia resulted. In the rabbits infected intranasally, from three to five instillations were given at intervals of from one to two days, while in the rabbits infected subcutaneously, three inoculations of 2 c.cm. of neutralized mixture were given at intervals of four days.

In the experiments here recorded it was desired to approximate as closely as possible to the conditions applying to routine vaccinations in man. In place of the more active neurovaccine, therefore, ordinary glycerinated calf lymph was used as a source of virus, while the immunizing mixture was inoculated intradermally, four injections being given at the same time, not at intervals of some days.

The hyperimmune serum was prepared in the rabbit by intravenous injections of vaccinia derived from rabbit testicle. Preliminary experiments were undertaken to determine the minimum dilution of the serum which was capable of neutralizing a suspension of vaccinia virus diluted 1 in 20 in saline. The suspension of the vaccinia virus was prepared by adding physiological saline to glycerinated calf lymph, centrifuging for ten minutes to throw down gross particles, and pipetting off the supernatant fluid. Virus suspension, 0.1 c.cm., was then mixed with an equal volume of the immune serum and inoculated intradermally. The highest dilution of the immune serum capable of preventing the development of any local lesion was, as a rule, 1 in 30; though in some rabbits dilutions of 1 in 40 protected, in others dilutions of 1 in 25 only were effective. For the development of immunity by means of a mixture of virus and immune serum, the final dilution of the immune serum selected was 1 in 20, as in six experiments this dilution prevented the development of any local lesions. On the other hand, with lower dilutions experiments showed that the final immunity obtained was much less, while when too high a dilution of serum was used the virus inoculated produced typical vaccinia reactions in the skin, although these lesions were generally less extensive than those produced by the same amount of vaccinia in normal rabbits.

The following is a typical protocol of a successful experiment, in which the quantities of vaccinia virus and immune serum were correctly adjusted.

January 5th, 1931.—Rabbit 117 received four intradermal inoculations of 0.1 c.cm. of a suspension of vaccinia virus diluted 1 in 10 in saline + 0.1 c.cm. of antivaccinia immune

serum diluted 1 in 10 in saline. The virus suspension was mixed with the immune serum immediately before the injection.

Rabbit 118 received four intradermal inoculations of 0.1 c.cm. of antivaccinia immune serum diluted 1 in 10 in saline + 0.1 c.cm. of saline.

Rabbit 119 received four intradermal inoculations of 0.1 c.cm. of a suspension of vaccinia virus diluted 1 in 10 in saline + 0.1 c.cm. of saline.

Rabbits 117 and 118 showed no local reaction and no significant rise in temperature. Rabbit 119 developed four typical vaccinal papules.

Five weeks later the three rabbits and a normal control were inoculated intradermally on fresh areas of skin with suspensions of 0.1 c.cm. of glycerinated calf lymph in saline in dilutions of 1 in 10, 1 in 100, 1 in 1,000, and 1 in 10,000.

Rabbits 117 and 118 showed slight hyperaemia eighteen hours later, but no reaction after forty-eight hours; in Rabbit 119 there developed typical vaccinal lesions, except that in a dilution of 1 in 10,000 the reaction was less extensive than in the control.

Similar results were obtained when the period between immunization and the test inoculation was extended to eight weeks.

In a further series of experiments the effect of injecting the immune serum and the virus suspension into different skin areas about 2 inches apart was tested. In order to inhibit the local reaction, however, it was necessary to use a lower dilution of the immune serum, a dilution of 1 in 5 being found suitable. Here again it was possible, without causing any local reaction, to produce by the injection of immune serum and vaccinia virus a degree of immunity equal to that produced by the injection of the same amount of living unneutralized virus.

IMMUNIZATION AGAINST YELLOW FEVER BY IMMUNE SERUM AND YELLOW FEVER VIRUS

It is well known that an attack of yellow fever is followed by a high degree of immunity, and Stokes, Bauer, and Hudson (1928) showed that even 0.1 c.cm. of the serum of a patient who had suffered from a severe attack of the disease was sufficient to protect a rhesus monkey against fatal doses of infected blood and the bites of infected mosquitos; but this passive immunity, produced by the injection of convalescent serum, seems to disappear within a comparatively short time. For example, Marchoux and Simond (1906) found that this protection began to disappear about the twenty-sixth day, and Burke and Davis (1930) record three laboratory cases of yellow fever in which injection of convalescent serum had been given four months, forty days, and twenty-four days respectively before the onset of the disease.

Although all three patients recovered, yellow fever shows such wide variations in its virulence that it is impossible to be certain that recovery was due to the serum. The last patient, who had been injected with 16 c.cm. of human convalescent serum only twenty-four days previously, had the mildest attack, and it seems probable that in his case the serum had some effect, but it is obvious that this method of protection has very limited applications. Apart from the method of immunization, to which we again draw attention in the present publication, the most valuable use of convalescent serum is for the treatment of individuals who have been exposed to infection. In the course of our experiments one of the laboratory attendants, who had certainly been inoculated accidentally with a virulent strain of yellow fever, received an inoculation of 20 c.cm. of human immune serum the following day, and showed no signs of the disease. On the other hand, the inoculation of immune serum after the symptoms have developed seems to have little effect on the course of the disease, as evidenced by the records of various laboratory infections, and also in the treatment of infected monkeys (Hindle, 1929).

The duration of passive immunity in monkeys seems to be of similarly short duration to that in human beings, as shown by the following experiments:

May 8th, 1929.—Rhesus No. 205, inoculated subcutaneously with 0.5 c.cm. citrated blood of an immune monkey, VH 30. Thirty-six days later it was inoculated subcutaneously with 0.05 gram of infected liver and spleen of Monkey 210, and had a non-fatal attack of fever. It was subsequently immune, and showed no reaction when inoculated with virus three months later.

May 8th, 1929.—Rhesus No. 206, inoculated subcutaneously with 1 c.cm. citrated blood of three immune monkeys, VH 9, VH 12, and VH 16. Thirty-six days later it was also inoculated subcutaneously with 0.05 gram of infected liver and spleen of Monkey 210, and died of typical yellow fever after seven days.

In these experiments it will be noticed that in one case (No. 205) some degree of protection may have persisted for thirty-six days, but in the other (No. 206) the immunity had completely disappeared after the same interval. It is evident, therefore, that convalescent serum is of little value for general prophylactic use "unless followed by a dose of active virus" (Hindle, 1929, p. 420).

On the other hand, the inoculation of a mixture of virus and immune serum seems to produce a more lasting immunity, as shown in the following experiments:

January 12th, 1929.—Rhesus No. 155, inoculated subcutaneously with 3 c.cm. of a mixture of human immune blood with 0.1 gram of infected liver. The monkey showed no febrile reaction during the following ten days, while a control animal inoculated with 3 c.cm. of a mixture of normal human blood with 0.1 gram of the same infected liver died of yellow fever six days later. Rhesus No. 155 was inoculated with large doses of virus on April 29th, 1929, and again on September 13th, 1929, but showed no fever or any other signs of infection.

November 3rd, 1928.—Rhesus No. 117, inoculated subcutaneously with a mixture of 5 c.cm. of human immune serum and 0.1 gram of infected liver. The mixture was allowed to stand fifteen minutes at room temperature before being inoculated. The monkey showed no signs of fever—either after this inoculation, or after four subsequent inoculations of large doses of virus (on two occasions as much as 3 grams of infected liver or spleen)—on November 14th, November 25th, and December 30th, 1928, and January 7th, 1929, respectively.

January 21st, 1930.—Rhesus No. 306, inoculated subcutaneously with a mixture of 4 c.cm. citrated blood of a patient who had had an attack of yellow fever twenty-four years previously, together with 0.05 gram of infected liver and 0.5 c.cm. of infected blood. This monkey showed no signs of fever, either after this inoculation or after a subsequent intraperitoneal inoculation of 1 c.cm. infected blood on March 6th, 1930.

March 14th, 1930.—Rhesus No. 316, inoculated intraperitoneally with 5 c.cm. of a mixture of 10 c.cm. hyperimmune monkey serum with 1 gram of infected liver. The mixture was left at room temperature for eighteen hours, and then 5 c.cm. were inoculated into the monkey. The animal showed no reaction after this inoculation, and on April 23rd, 1930, was again inoculated with 1 c.cm. of virulent blood without showing any fever.

In this experiment the immune serum had had no action upon the virus *in vitro*, for 5 c.cm. of the mixture was centrifuged after it had been standing for eighteen hours, and the sediment was washed three times in a mixture of normal saline and ascitic fluid to remove the immune serum. The washed sediment was then inoculated into a monkey (No. 317), which died six days later of typical yellow fever.

It is unnecessary to multiply these examples, which are supported by the independent investigations of many observers. The evidence proves conclusively that the simultaneous injection of immune serum and virus in monkeys produces no obvious ill effects, and, moreover, is followed by a lasting immunity, in marked contrast to the

comparatively short duration of the passive immunity produced by the inoculation of immune serum alone.

DISCUSSION

The use of virus and immune serum for immunization against virus diseases has many analogies with the use of a mixture of toxin and antitoxin in immunization against diphtheria. That such a toxin-antitoxin mixture is slowly dissociated was shown by Arrhenius and Madsen (1902), who found that free toxin was liberated from the mixture so gradually, and in such small amounts, that immune bodies were produced in the absence of disease symptoms. Glenny and Pope (1925) have also pointed out that diphtheria toxoid, when injected subcutaneously, will immunize rabbits, but may fail when given intravenously. If, however, the toxoid be partially neutralized by antitoxin, rabbits can be immunized by the intravenous route. This is apparently due to the fact that there is too rapid an elimination after intravenous injection, while the presence of antitoxin in the mixture may delay absorption or elimination. Glenny (1925) therefore concluded that the efficiency of toxin-antitoxin mixtures depends on the slow dissociation of the toxin from the antitoxin, so that the animal injected is constantly stimulated. Now Todd (1928), in the case of fowl pest, and Andrewes (1928), in the case of vaccinia, have shown that dilution of an inactive virus-immune-serum mixture renders the mixture active. The analogy with toxin-antitoxin mixture is obvious. It seems probable, therefore, that a virus-immune-serum mixture, when injected into an animal, gives off virus very slowly, with the result that the active virus is insufficient to give rise to symptoms of disease, but sufficient to produce active immunization.

In the case of vaccinia, the use of a mixture of immune serum and virus would seem to have advantages over the method of vaccination now generally employed, provided that the relationship between the titre of the immune serum and the virus is correctly adjusted.

The fact that a virus-immune-serum mixture has given satisfactory results in two virus diseases so dissimilar as vaccinia and yellow fever suggests that this method may have a very general application in other diseases due to ultra-microscopic viruses.

CONCLUSIONS

Experiments are recorded showing that immunization against vaccinia and yellow fever may be obtained by the injection of a mixture of immune serum and living virus, without giving rise to any general or local reaction.

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