***On the one hand are the high hopes stirred by a growing number of sanguine people who predict a poliomyelitis vaccine "just around the corner." On the other are our memories of early and unsuccessful efforts at polio prophylaxis, matching more recent difficulties with influenza vaccines. Just where do we stand? If you want a clear picture of current perplexities, and rising hopes, then this paper becomes a "must" for you.***

# Principles of Immunization as Applied to Poliomyelitis and Influenza*

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WITHOUT entering upon a discussion of the question of the mechanism whereby antibody is effective in the prevention of the microbial diseases, let us make the now reasonable assumption that the relationship between antibody and resistance is more than one of mere association—that it is one of cause and effect. It would follow from this that it would be desirable to raise and maintain the level of serum antibody above a critical threshold, because in so doing it may be expected that immunity will result. From evidence already available for influenza and poliomyelitis it is clear that the question which really concerns us is not whether this is so, but rather *how* it may be accomplished. It is the purpose of this presentation to attempt to answer this question.

It has long been known that antibody formation occurs, not only as a result of an infectious process which is accompanied by symptoms of disease, but following asymptomatic infections as well. Moreover, it has been shown that antibody formation may also be induced when a properly constituted preparation of a toxin, modified to be free of danger and called a toxoid, is injected, or, if a suitably killed bacterial or viral suspension is similarly employed. Thus, it would appear that the natural methods for inducing immunity may be simulated by exploiting one or more approaches based upon these fundamental considerations.

One approach, which is referred to as the method of variolation is the first that was employed in this way. Since it is based upon the principle of infection induced with an essentially nonpathogenic relative of the agent in question, such a method closely resembles the natural phenomenon. Some consider this to be a better approach than one in which immunity is induced by methods which do not employ infection by a living agent. However, there is evidence to suggest that it may be possible to improve upon natural methods of immunization.

It is to be recalled that variolation

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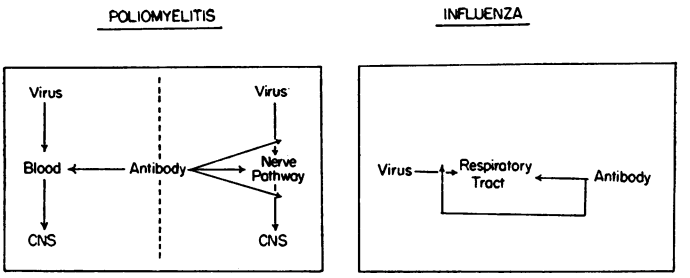
FIGURE 1

SITES OF MEDIATION OF EFFECT OF ANTIBODY
IN THE PREVENTION OF POLIOMYELITIS AND INFLUENZA



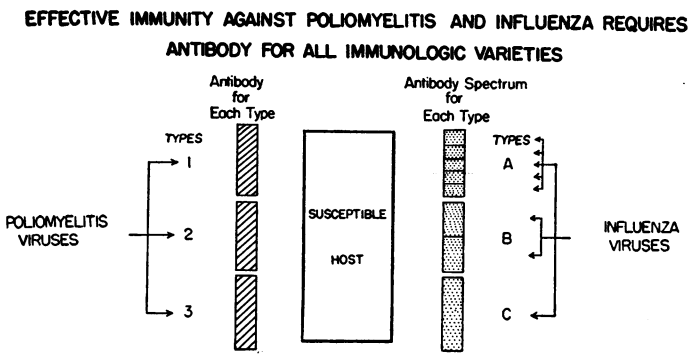

was employed before the science of immunology was born and, in the face of dangers with overwhelming odds, there was no other choice available at that time. The fact that poliomyelitis is a disease that can cause crippling would make it seem that even at great cost it is necessary to approach the solution, to the problem of this disease at least, without subjecting any human being to more than reasonable hazard. Although the risk of influenza continues and the risk of paralytic poliomyelitis is increasing rapidly, the advances in our knowledge of immunology and immunologic mechanisms and the development of the means for inducing the immune state artificially have so progressed that these dangers may well be overtaken by procedures that do not entail the risk of acquiring the disease in the course of attempting to induce a state of immunity.

## IMMUNOLOGIC CONCEPTS

*Sites of Mediation of Antibody Effect*—The ways in which antibody may function in the prevention of paralytic poliomyelitis, or influenza, are illustrated diagrammatically in Figure 1. It may be seen that for the antibody to be effective in intercepting the virus of poliomyelitis, before invasion of the central nervous system, it would be necessary that this take place either in the bloodstream, if this is the avenue to the central nervous system, or that antibody be able to intercept the virus as it may move along nerve pathways. If the recently demonstrated viremia stage represents a cónstant occurrence before invasion of the central nervous system, then relatively little antibody would be necessary to intercept the virus on its way to the centers where the irreversible damage is done. On the other hand, it has been amply shown—directly in experimental animals and, indirectly, in human subjects—that the virus of poliomyelitis may at times proceed along nerve pathways. It is evident that in one case less antibody would be required than in the other. We shall return to this question again later.

In influenza, where the virus attacks the surface epithelium of the respiratory tract and the antibody present in the blood is on the opposite side of this membrane, it is evident that spread of infection may be arrested by the antibody contained in inflammatory exudate. If the level of antibody in the serum is high it may overflow in sufficiently high concentration into the secretions that bathe the surface of the respiratory tract, and, thereby, prevent primary invasion of the susceptible epithelium by the influenza virus. Thus,

FIGURE 2




for antibody to be effective it must be present where it may come into intimate contact with the virus *before* invasion of the susceptible cells.

*Specificity of the Immune Mechanism* —Another important consideration for effective immunity against poliomyelitis and influenza, each of which is really a family of diseases, is the necessity for creating an antibody barrier for all immunologic varieties. This is illustrated in Figure 2 where it may be seen that the susceptible host will be spared the ravages of these diseases only if there is a solid wall consisting of antibody for each type of poliomyelitis virus and for each of the types and subtypes of influenza virus. For the control of poliomyelitis in this way, the problem may be relatively simple in that there appears to be a high degree of homogeneity within each type.

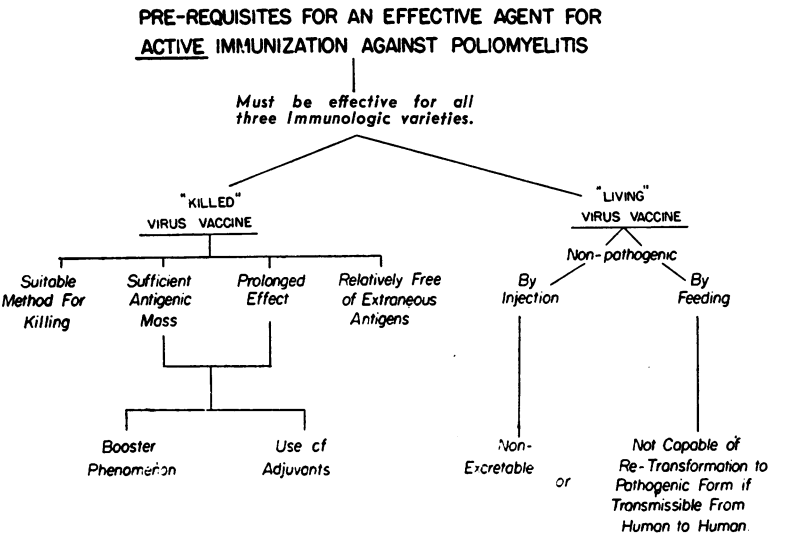
For influenza the problem is more complex in that there is a rather wide degree of heterogeneity, among type A strains, at least, the full extent of the variation of which is as yet unknown. The fact that influenza A epidemics occur more frequently than epidemics of influenza B—at intervals of two to three years as compared to five to seven years—may well be because of the greater degree of heterogeneity of the A varieties as compared with the B. The virus of influenza C, which has been recognized only recently, appears to be different in many respects from the viruses of influenza A and B. Although a member of the influenza family of viruses, the epidemiologic behavior of this agent is different from that of the other two viruses, and it is conceivable that immunologic homogeneity may be one reason for the apparent absence of influenza C as a major cause of periodic eruptions.

## REQUIREMENTS FOR A POLIOMYELITIS VACCINE

It is clear from the foregoing illustrations that fundamental immunologic principles that apply equally well to influenza and poliomyelitis do so in different ways. Nevertheless, an approach which has been successful in part, and is being improved upon, for the control of influenza has served to facilitate the progress of the problem of immunization against poliomyelitis. Because this progress is so much less advanced, it is desirable to dwell briefly upon the essential requirements for a preparation that may be employed to induce immunity to poliomyelitis. A summary of prerequisites for the two principal approaches, either a killed-virus vaccine or a living-virus vaccine is presented in Figure 3.

The first consideration for a living virus vaccine is that it must be devoid

FIGURE 3




of the capacity to induce the paralyzing disease in man. Preparations containing live-virus may be administered either by feeding or by injection. If fed, it is likely that the agent will appear in secretions or excretions; under such circumstances the virus may be transmissible from man to man. It will be necessary, therefore, to be certain that the originally nonpathogenic virus is not capable of retransformation to the pathogenic form after undergoing human passage. If a living-virus vaccine is given by injection, the same prerequisites would apply if the virus, so administered, is found in the secretion of the oropharynx or in the contents of the bowel.

A killed-virus vaccine poses no problems of safety, with respect to the virus component, if treatment is carried out in a way which will destroy completely the capacity to multiply without impairing antigenic effectiveness. For this it is necessary that there be available a sufficiently rich source of virus relatively free of extraneous antigens, and that there is a reliably reproducible method for destroying infectivity without totally destroying antigenicity. Moreover, it would be desirable, from the practical viewpoint, that means be available for inducing an adequately prolonged effect and one that may be readily reinforced when needed.

The development of tissue culture methods for the propagation of poliomyelitis viruses has yielded preparations that are relatively free of extraneous antigens, and the solution to the problem of adequacy of antigenic mass is being approached rapidly by greater proficiency in cultivation of these viruses. The use of precise methods for studying the effect upon the virus of different methods of inactivation has provided a means, that is reproducible, for destroying infectivity without eliminating antigenicity. The problem of sufficiency of antigenic mass and adequate prolongation of immunizing effect can both be influenced by the application of the "booster phenomenon" and by the use of mineral oil adjuvants; both will be considered in more detail in the discussion to follow.

FIGURE 4

**DISSOCIATION OF PARALYTOGENIC AND ANTIGENIC EFFECTS BY DILUTION AND INJECTION VIA A NON-NEURAL ROUTE**

| | | Dilution of Tissue Culture Fluid | | | | | |
|---|---|---|---|---|---|---|---|
| | | $10^{0}$ | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ |
| MAHONEY (Type 1) | Paralysis | + | + | + | + | + | 0 |
| | Antigenicity | + | + | + | + | + | 0 |
| MEF-1 (Type 2) | Paralysis | + | 0 | 0 | 0 | 0 | 0 |
| | Antigenicity | + | + | + | + | + | 0 |
| SAUKETT (Type 3) | Paralysis | + | 0 | 0 | 0 | 0 | 0 |
| | Antigenicity | + | + | + | + | + | 0 |

*Studies in cynomolgus monkeys injected intramuscularly with fluids emulsified with mineral oil.*

Although the approach of most investigators interested in the question of a live-virus vaccine for poliomyelitis is along lines that involve administration of such preparations by feeding, our interest in exploring this question has been limited to the *injection* rather than to the *feeding* of such preparations to experimental animals. In this way an attempt has been made to study the factors responsible for the subsequent occurrence of immunity, with or without paralysis, following the injection of live-virus. It is well known that differences exist among strains of poliomyelitis virus with respect to their virulence, or pathogenicity, when administered via the intracerebral route in monkeys.

Differences of even greater degree among strains have been observed in respect to their paralyzing effect when administered by a non-neural route. Figure 4 summarizes the results of a series of experiments designed to determine whether or not paralysis and antibody formation occur when cynomolgus monkeys are injected intramuscularly with different dilutions of tissue culture fluids containing selected strains of the three virus types. It is clearly evident from this chart that the Mahoney strain of type 1 virus is highly paralytogenic; even at the highest dilution that is capable of inducing antibody formation it is possible for paralysis to occur. For the MEF-1 and Saukett strains of types 2 and 3 virus, respectively, it appears that paralysis does not result from the injection even of large amounts of living virus. It may well be that differences in the characteristics of invasiveness for the central nervous system may be the reason for the difference in behavior of different strains. However, it is also conceivable that a longer incubation period for the MEF-1 and Saukett strains, as compared with Mahoney, may allow antibody to appear before the central nervous system is invaded. Although the reasons for the dissimilar behavior of these strains is not understood, that they are different is clear indeed.

The information presented in Figure 4 is not intended to suggest that all type 1 strains behave like Mahoney; in fact, this strain is unusual in this respect, and most other type 1 strains are similar to the types 2 and 3 viruses selected for

this illustration. Should the present attempts at developing a long-lasting immunity with a killed-virus vaccine fall short of expectations, it would seem highly probable that a procedure based upon the findings in Figure 4 may well succeed if strains are properly selected. This method has been under investigation for several years and it is possible to say that two years after inoculation antibody is present at an undiminished level from the peak obtained shortly after immunization of rhesus monkeys.

Because of the indeterminate risk attendant upon the use in man of a live-virus vaccine for a disease such as poliomyelitis, our principal attention has been directed toward exploring the possibility that a noninfectious preparation might be effective in preventing paralysis. The principal objective in these studies has been to determine the conditions required for inducing antibody formation in a consistent and reproducible manner. A second and longer range objective is to determine how antibody so formed may be made to persist or be capable of being recalled quickly and in sufficient concentration to be effective in preventing paralysis even if infection occurs at some time subsequent to immunization.

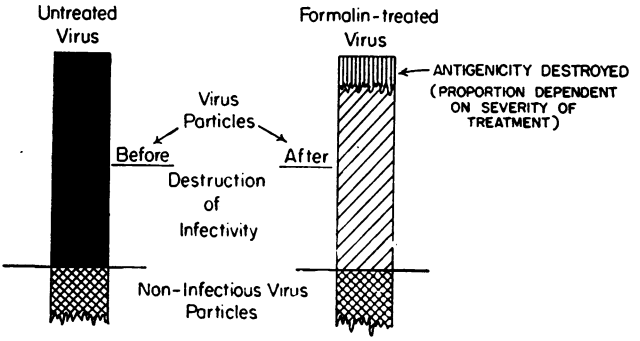
As indicated above, there are several prerequisites that must be satisfied before one should consider realistically the preparation of a noninfectious virus vaccine for poliomyelitis. Let us consider these in more detail.

*Probable Existence of Occult Antigenic Material in Fluids from which Vaccines Are Made*—One of the prerequisites that must be met is the availability of a sufficient mass of preformed virus to be effective in inducing antibody formation without requiring virus multiplication to provide the antigen needed for an adequate stimulus. The possibility that a sufficient concentration of virus was present in the available tissue culture fluids was based upon the concept illustrated diagrammatically in Figure 5. The solid black bar represents virus particles that are fully infectious; however, it is believed that in addition to virus particles capable of causing disease there are also present in such fluids, particles which may be noninfectious but which are still antigenic.

Several years ago the probable existence of virus particles capable of behaving in these two different ways was demonstrated in experiments employing suspensions of CNS tissue from para-

FIGURE 5

PROPORTION OF INFECTIOUS TO NON-INFECTIOUS VIRUS PARTICLES IN VIRUS SUSPENSION BEFORE AND AFTER TREATMENT WITH FORMALIN



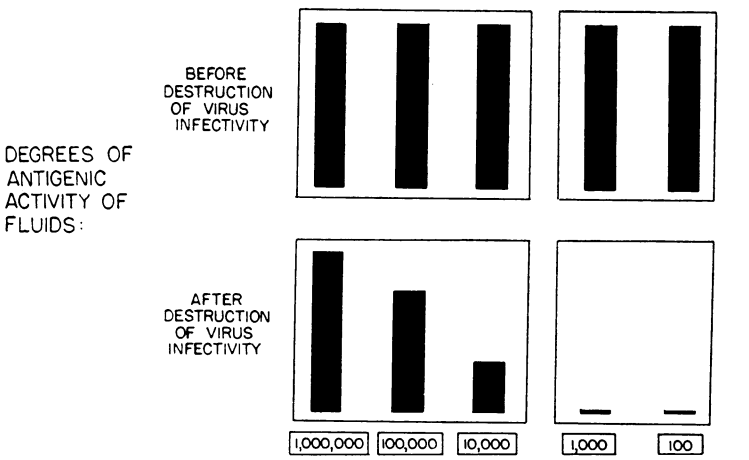
lyzed animals. Although we do not yet possess methods for direct measurement of the relative proportion of infectious and noninfectious virus particles in a mixture of the two varieties, it does appear that the preparations which have been employed in the course of our recent investigations in monkeys and in man have possessed a degree of antigenic activity greater than that which would have been anticipated on the assumption that no more than the amount of virus revealed by the most sensitive infectivity measurements was present. If this concept is valid, it would serve to explain, in part, why it has been possible to demonstrate the antigenic effectiveness of virus preparations which possess such relatively low infectious titers as compared with virus-containing fluids required for the preparation of vaccines for Japanese B encephalitis, influenza, and certain other virus diseases.

When the living virus preparation is treated chemically, the infectious activity is destroyed. Under such circumstances it is to be expected that the antigenic activity of both the infectious and the noninfectious particles might be impaired. Since the degree of destruction of antigenic activity would depend upon the severity of the chemical treatment, it would appear, as illustrated schematically in Figure 5, that the quantity of effective antigenic mass that remains after chemical treatment would depend upon two factors: (1) the total antigenic mass in the starting material, which consists of both infectious and noninfectious virus particles; and (2) the proportion of each that remains after chemical alteration for destruction of infectivity. The importance of both will be illustrated in the figures to follow.

*The Importance of Antigenic Mass*—The relationship which has been observed between virus concentration and

FIGURE 6

**ILLUSTRATION OF RELATIONSHIP BETWEEN VIRUS CONCENTRATION AND DEGREE OF ANTIGENIC ACTIVITY OF FLUIDS CONTAINING POLIOMYELITIS VIRUS**

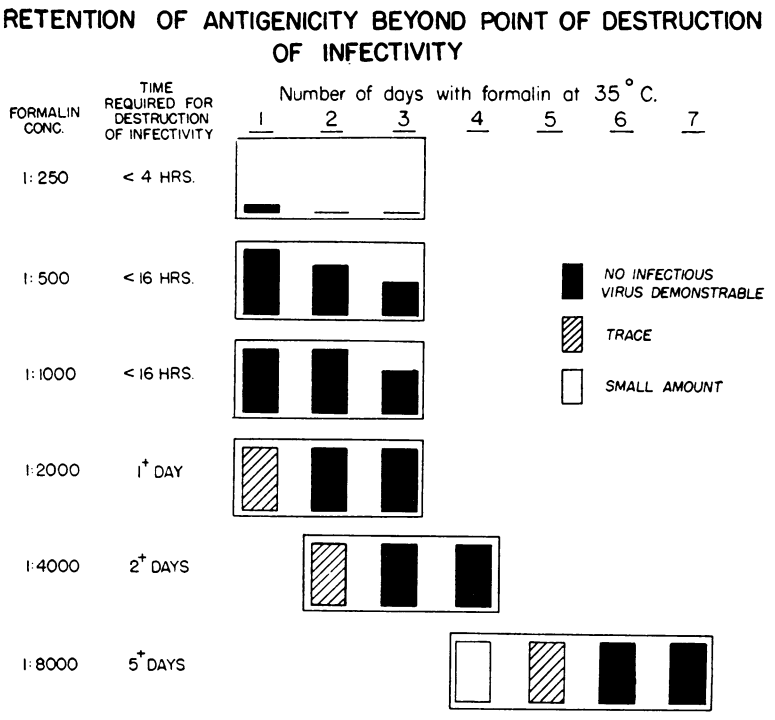



*Estimated conc. of virus in fluid used for preparation of vaccine. (hypothetical no. of virus particles.)*

degree of antigenic activity of fluids containing the poliomyelitis virus, before and after destruction of virus activity, is illustrated in Figure 6. The height of the vertical bars represents the degree of antigenic activity of vaccines prepared with different concentrations of virus. The numbers representing virus particles are hypothetical rather than actual and are based upon the approximate number of infectious units for tissue culture. Figure 6 has been divided into four frames: the two at the right illustrate the relationships that are observable when comparisons are made between living and dead virus preparations containing an antigenic mass too little to be effective without virus multiplication; the two frames at the left illustrate the effects observed with preparations of increasingly higher virus concentration after gentle treatment with formalin for destruction of infectivity. From the upper portion of the chart it is evident that infectious virus exerts a uniform antigenic effect. This is clearly the result of infection which, through virus multiplication, affords the full antigenic stimulus. The lower portion of the figure illustrates the gradation of effect resulting from injection of virus preparations which are not capable of multiplication; the response to such preparations can only be to the antigenic mass introduced in the inoculum.

*Effect of Chemical Treatment Upon Antigenic Activity*—A large number of experiments has been performed to define the optimal conditions for destruction of infectious activity, with maximal

FIGURE 7




*Height of columns indicates approximations of degree of antigenic effect induced in monkeys with two doses of fluid emulsified with mineral oil.*

FIGURE 8

RELATIONSHIP OF SIDE-EFFECTS TO COMPOSITION OF ADJUVANT MIXTURES

| VIRUS IN SUSPENSION OF CNS TISSUE AND MINERAL OIL PREPARED AS AN EMULSION WITH | POTENTIATION OF ANTIBODY FORMATION | UNDESIRABLE LOCAL REACTION | ALLERGIC ENCEPHALO-MYELITIS |
|---|---|---|---|
| Standard Arlacel A + Acid Fast Bacilli | ++ | ++ | ++ |
| Standard Arlacel A | + | ± | 0 |
| Purified Arlacel A | + | 0 | 0 |

retention of antigenic activity. The observations made are summarized in Figure 7. The concentration of formalin used for preparation of each experimental vaccine is indicated in the first column and the second column indicates the time required for destruction of infectivity; the remainder of the chart shows the relative degree of antibody response induced in monkeys with fluids treated with the different concentrations of formalin at 35° C. for the number of days indicated. It is evident that an excess of formalin destroys antigenicity and that an appreciable margin exists between the time at which infectivity is destroyed completely and antigenicity begins to be diminished, or is destroyed completely. This is true only if there is sufficient concentration of antigenic substance before chemical treatment is applied. If the concentration of virus in the starting material is inadequate, then the margin between destruction of infectivity and elimination of antigenicity is narrowed or reduced entirely, as was illustrated in Figure 6.

USE OF A MINERAL OIL ADJUVANT FOR ENHANCING ANTIGENIC EFFECTIVENESS

The need has long been recognized for a method of enhancing the immunizing effect of vaccine preparations that are of borderline effectiveness and for prolonging the effect of those that are of established value. It is for this reason that considerable attention has been devoted by many investigators to an exploration of means whereby this may be achieved. One of the most effective methods that has been employed is that of emulsification with mineral oil, as devised by Dr. Jules Freund. The single hindrance to the clinical applicability of this phenomenon was the occurrence of undesirable side effects at the site of inoculation. It seems that it should be possible to obviate these in one way or another. In the course of investigating the possible usefulness of this method for influenza virus vaccines, considerable progress has been made toward this end. As a result of rather extensive experience in animals, and even more extensive observations in human subjects—now including more than 30,000 persons—it is possible to present, tentatively, a summary of the relationship between the occurrence of side effects and the composition of the adjuvant mixture. These relationships are shown in Figure 8.

To those who have worked with emulsified vaccines it is well known that the

original Freund adjuvant mixture, which contained acid-fast bacilli, produces marked potentiation of antibody formation but it also produces marked local reactions, and if the emulsion contains CNS tissue a demyelinating encephalomyelitis will also result. However, when acid-fast bacilli are omitted from the emulsion mixture, there is potentiation of antibody formation which is somewhat less marked than with the acid-fast organisms; undesirable local reactions may or may not occur, and the CNS tissue without the acid-fast bacilli has not produced encephalomyelitis in more than a thousand monkeys inoculated repeatedly with such mixtures. Thus, the omission of the acid-fast bacilli appears to eliminate the risk of organ-damage or autosensitization to tissue that might be present in the vaccine mixture. Further studies have revealed that by purification of the emulsifying agent potentiation of antibody formation is still retained and undesirable local reactions appear to have been eliminated. With such preparations it will now be possible to explore the application of this principle further to determine whether or not any hidden and as yet unobserved pitfalls exist in the utilization of this highly efficient method for enhancing and prolonging antibody formation.

Dr. Freund had shown that various antigens emulsified with mineral oil in a water-in-oil emulsion, with or without acid-fast bacilli, will enhance the height and persistence of antibody formation. In our studies employing this phenomenon, using influenza virus vaccines both in experimental animals and in man, it has been found that quantities of antigenic material that are ineffective when administered as an aqueous preparation are substantially effective when properly emulsified (Figure 9).

FIGURE 9

ANTIBODY RESPONSE TO DIMINISHING QUANTITIES OF INFLUENZA VIRUS VACCINE EMULSIFIED WITH MINERAL OIL OR IN SALINE

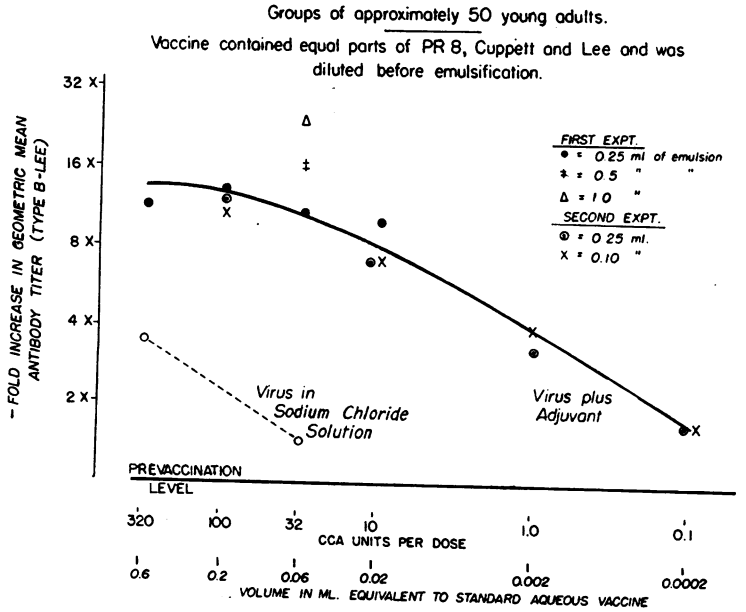
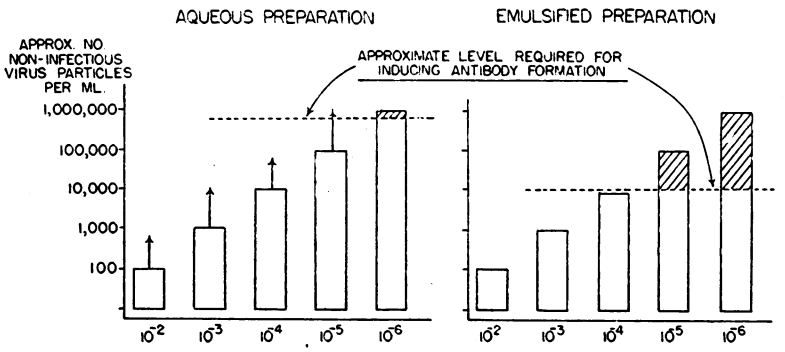


FIGURE 10

**QUANTITY OF VIRUS REQUIRED FOR PREPARATION OF A NON-INFECTIOUS VACCINE**




*Infectious Titer of Tissue Culture Fluids Used For The Preparation of A Non-Infectious Vaccine*

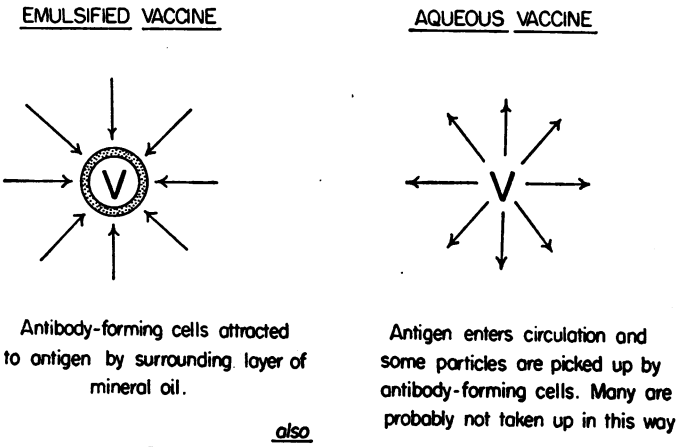
† *Indicates limits of practicable range of further conc. of virus that it is possible to achieve by physical or chemical methods.*

This observation has been extended in studies with poliomyelitis virus vaccines and Figure 10 illustrates, perhaps in not too simple a fashion, the difference between the quantity of virus required for the preparation of a noninfectious vaccine both with, and without, emulsification with mineral oil. The dotted line, situated at two levels, indicates the approximate level of virus required for inducing antibody formation if an aqueous preparation is employed or if the antigen is fortified by emulsification with mineral oil. It should be clear from these relationships that preparations of poliomyelitis virus as well as influenza virus which may contain an insufficient quantity of antigen if used as an aqueous preparation may be rendered effective if emulsified with mineral oil. The arrows above the vertical columns indicate the practicable limits for concentration of virus that it is possible to achieve by physical or chemical methods. Other approaches for increasing antigenic mass which are still to be explored, before the limits of virus concentration that can be achieved are established, involve manipulation of the system in which virus multiplication takes place. Thus, as an adjunct to methods which will provide a sufficient antigenic mass for an effective vaccine, it appears that emulsification with mineral oil may be most useful.

The mechanism for the improved effectiveness of vaccines emulsified in mineral oil is illustrated schematically in Figure 11. The virus emulsified in mineral oil is contained in the aqueous phase of the water-in-oil emulsion; thus the antigen is surrounded by a thin film of mineral oil which attracts cells that appear to be important in antibody formation. In this way the antigen is more likely to be brought into direct contact with an antibody forming cell than is the antigen in an aqueous preparation; the latter is absorbed into the circulation and distributed in such a way that some particles are picked up by antibody forming cells and many are probably not taken up in this way. Thus, it would appear that apart from creating

FIGURE 11

**RELATIONSHIP OF ANTIBODY FORMING CELLS TO VIRUS EMULSIFIED WITH MINERAL OIL OR TO VIRUS IN AN AQUEOUS MENSTRUUM**




what might be referred to as an antibody forming organ at the site of inoculation, there is a more efficient utilization of the injected antigen.

The efficiency of utilization of the injected antigen is further enhanced by the elimination from the inoculum of extraneous antigenic constituents which have characterized most virus vaccines prepared from tissue suspensions. Experimental verification of this has been obtained with many systems and has been further substantiated in our studies with influenza virus vaccines and poliomyelitis virus vaccines emulsified with mineral oil adjuvants. Figure 12 illustrates schematically how direct contact between antibody forming cells and virus particles are possible with suspensions of relatively pure virus in tissue culture fluids; whereas a mixture of virus and other particles in crude tissue suspensions result in a competition between the specific virus and other particles for space in the antibody forming system.

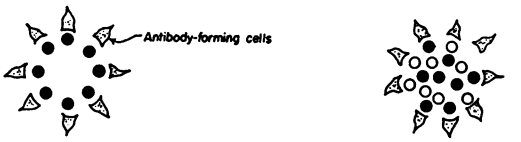
In addition to the emulsification increasing the efficiency of utilization of the injected antigen, there is an advantage in the increased antibody levels which are not readily attainable with the aqueous antigen and the advantage of greater persistence of antibody. Whether or not these benefits can be utilized will depend upon the continued favorable clinical observations following the inoculation of emulsions made with purified reagents.

### PERSISTENCE OF CLINICAL IMMUNITY

If it is assumed that clinical immunity is linked, in part, to the level of serum antibody, then it would follow that the interval through which antibody is present in an amount above a critical threshold would determine the length of time that immunity will persist. A priori, it would appear that the mode of path-

FIGURE 12

MECHANISM OF INTERFERENCE WITH ANTIBODY FORMATION FOR A SPECIFIC VIRUS BY PRESENCE OF EXTRANEOUS TISSUE ANTIGENS




Suspension of relatively pure virus in tissue culture fluid

Mixture of virus and other particles in crude tissue suspension

ogenesis of the particular disease in question would determine the level of antibody required for a state of immunity to prevail.

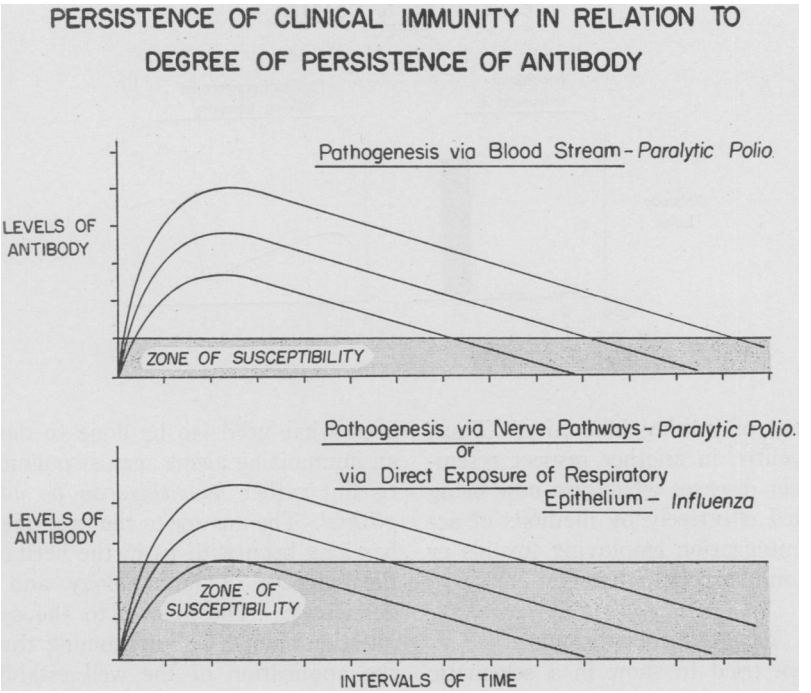
On the basis of this assumption the schematic representations in Figure 13 are presented to indicate the difference in the level of antibody required for a disease in which virus is transported via the blood stream to the central nervous system or directly invades the susceptible cells of the respiratory tract, in influenza, or of the nervous system, in poliomyelitis. If the pathogenesis of paralytic poliomyelitis is via the blood stream, then relatively small quantities of antibody would be necessary to intercept the virus before invasion of the central nervous system. On the other hand, if the virus of poliomyelitis is transported from the portal of entry to the central nervous system directly over nerve pathways, it would be necessary that there be present higher levels of antibody in the circulating blood, sufficient to overflow into the perineural channels and perhaps intercept virus invasion in that way.

The pathogenesis of influenza in relation to the way in which antibody might be effective would resemble the latter example for poliomyelitis. The level of serum antibody would have to be sufficiently high so as to be present in adequate concentration in the nasal secretions, as Dr. Francis has demonstrated, and in this way only can it serve to intercept the establishment of infection and disease. If the persistence of clinical immunity is related to the degree of persistence of serum antibody, it would follow also, from what is known about rate of antibody decline following immunization, that it would be influenced by the level to which antibody is raised initially. Thus, it should be possible to predict the probable duration of immunity from studies on the height to which antibody level is raised and the general course of its decline over a period of months after inoculation.

Although the assumption has been made that persistence of immunity is related to the presence of circulating antibody, it is well known that once an individual has been exposed to a particular antigen he will, on a subsequent occasion, react much more promptly with a great abundance of antibody and do so in response to much smaller amounts of antigen, than he did at the time of his first exposure.

Dr. Colin MacLeod, in his presidential address before the American Association

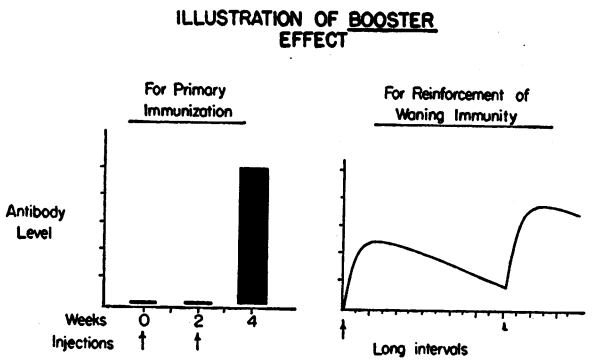
FIGURE 13




of Immunologists in 1952, presented an attractive theory to explain the presence or absence of lifelong immunity on the basis of the length of incubation period for the disease in question. He has postulated that the second attack of disease will be prevented, even in the absence of neutralizing antibody at the time of exposure, if the incubation period is longer than the three to seven days required for the "booster" response to the second antigenic stimulus. He has suggested also that the reason for the relatively short-lived immunity to such diseases as the common cold and influenza may be due to the decline in level of antibody with a return of the state of relative susceptibility which is not rapidly enough reinforced by the new exposure because of the shortness of the incubation period. Whether or not this phenomenon may be operative in producing what appears to be lifelong immunity to poliomyelitis, and may influence the persistence of immunity following artificial primary immunization, remains to be determined.

Nevertheless, sufficient information has been gathered in the course of our studies with poliomyelitis vaccines, both in experimental animals and in human subjects, to indicate that substantial levels of antibody can be induced if multiple inoculations are given for primary immunization and, furthermore, that on the declining limb of the antibody curve indicating waning immunity, inoculation of relatively small amounts of antigen is capable of calling forth very substantial levels of antibody. This is illustrated schematically in Figure 14. It would appear that for poliomyelitis the booster effect, with or without the mineral oil adjuvant, might well serve as a means for enhancing the efficiency of primary immunization and for rein-

FIGURE 14




forcement of waning immunity. Thus, poliomyelitis, in another respect resembles other diseases which are now being controlled effectively by methods of active immunization employing toxoids or other noninfectious antigens.

## CONCLUDING REMARKS

I have tried to show in a schematic and, perhaps, oversimplified fashion the way in which the well established principles of immunology may be applied to the development of active immunizing agents for the control of influenza and poliomyelitis. The experimental evidence that has permitted the illustrations drawn, has been published in part, but many unreported data are represented in the charts presented here.

The question, for a long time, has not been what needs to be done to develop an immunizing agent against poliomyelitis but rather *how this can be accomplished.* The answer to the first question has long been with us in the heritage of the sciences of immunology and epidemiology. The answer to the second question should be forthcoming through the application of the well established principles of these sciences. By utilizing the experience accumulated in the study of other diseases in which progress is further advanced, the solution to the problems that remain will be hastened. But, only through a synthesis of the ways of the laboratory investigator in the immediate and related fields of the clinical investigator and of the epidemiologist will the solution to the problem of poliomyelitis be forthcoming.