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VACCINATION AGAINST MEASLES: FRANCIS HOME REDIVIVUS

FOREWORD

This paper is an attempt to review in the light of earlier research certain recent work on measles virus with emphasis on the development of a living attenuated vaccine against the disease. It was presented as a Cameron Prize Lecture before the Faculty of Medicine, Edinburgh University, 16 May 1961. Since it has not been submitted for publication the author decided it might be appropriate to offer it as his contribution to this collection of papers that jointly testify to the great respect and affection which all of his colleagues, pupils, and friends have for John Paul.

Although written for another occasion it seemed fitting for several reasons to include it here. There is, among John Paul's earliest professional communications, one devoted to a pathological study of measles conjunctivitis. Throughout the years as epidemiologist and distinguished student of Preventive Medicine he has continued to maintain his interest in this ubiquitous and fascinating disease. Recently in John Paul's laboratory significant additions to our knowledge of measles have been made by several members of his staff whose investigations he warmly encouraged. Finally, as director and afterwards member of the Commission on Viral Diseases of the Armed Forces Epidemiological Board he has, by his helpful enthusiasm and wise counsel, contributed substantially to the studies in our own laboratory which are described in this paper.

INTRODUCTION

The problem of vaccination against measles is an old one, and only now may the solution be at hand. Its antiquity is accented by the fact that the first attempt to immunize against measles preceded by nearly half a century Jenner's discovery of smallpox vaccine. This first attempt was made in 1758 by Francis Home of Edinburgh. Home was then a Fellow of the Royal College of Physicians and, to judge by his many excellent

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case reports, an active practitioner in the City and in the Infirmary. Later he became Regius Physician and Professor of Materia Medica in the University.

In reading Home's prototypic experiments as well as some of his other medical writing. I have been impressed with the keenness of his powers of observation, the logic and modernity of his thinking, his experimental approach to clinical problems, and his healthy scepticism. These characteristics reveal the father of inoculation against measles as a somewhat neglected forerunner of the great age of experimental medicine that was to come in the next century.

Measles in Home's time appears to have presented a far more serious problem than it does nowadays, at least in the so-called "well developed" countries. In other parts of the world, however, as I shall later reemphasize it still frequently takes the form of severe illness with a mortality that is far from negligible. The gravity of the disease in mid-eighteenth century Scotland is well exemplified by Home's statement at the beginning of his book entitled "Medical Facts and Experiments" in which he described his work on vaccination. Here he remarks, in what to us today appears to be a monstrous understatement, that in the epidemic which occurred in Edinburgh about the beginning of December, 1758 the measles were "in general of a mild sort; and not about the twelfth part died of those who were attacked". Further on he implies that in former outbreaks the incidence of serious complications, especially that of pneumonia, as well as the mortality was much higher.

This disturbing situation led him to attempt inoculation of "morbilous matter" by a parenteral route in the hope, as he puts it, of rendering "the disease more mild and safe in the same way as the Turks have taught us to mitigate the smallpox". Home's theoretical basis for this procedure appears eminently logical. "I suspected strongly", he wrote, "that the cough, often so harassing, even in the mildest kind, was produced by receiving the infection mostly by the lungs; and I hoped that this symptom would abate considerably if I could find a method of communicating the infection by the skin alone".

At the outset Home was confronted with a major difficulty; for, unlike smallpox there was, as he says, "no matter to be had from the measles" that appeared suitable for inoculation. He goes on: "A woolen glove taken from the arm of a measly patient would not answer my purpose, as part of the infection might be drawn in by the lungs. I could not find a sufficient quantity of scaly matter, after the measles were dried, to serve my purpose". Characteristic of his intuitive correctness of thought was the way he

"o'er leapt" this hurdle: "I then", he declares, "applied directly to the magazine of all epidemic diseases, the blood". Not only in the choice of blood as inoculum but also in that of the optimal time for taking it he displayed much discernment: "As the measly matter behoved to be but a small proportion of the whole mass, I chused to make use of the blood when it contained the morbilic matter in the highest state of acrimony". He reasoned that the time of the "highest state of acrimony" or, as we should say, virulence, was at the peak of the fever, shortly after the eruption appeared. Experiments of the present century have justified Home's choice since they showed not only that viremia regularly occurs but that the virus present in the blood at the onset of the eruption soon thereafter disappears from the circulation. Noteworthy also are his conjectures that the amount of the virus in the blood is small and, as he points out later, may rapidly lose its infectivity if the blood is too long preserved before inoculation. These enlightened guesses have lately received ample experimental confirmation by investigators such as Ruckle and Rogers and ourselves, using the tissue culture method which I shall soon mention.

Home described the results of inoculation of measles blood in a group of twelve children. In ten of them an eruption that he considered characteristic of the disease was noted following a rather regular interval. Two of the oldest children, one of whom gave a history of measles, failed to present an exanthem. Rash, when it occurred, was usually preceded by fever, signs of conjunctivitis and coughing. These manifestations appeared most frequently six to seven days after inoculation and usually preceded the rash by one to three days. That he "might see the difference of the disease when it is communicated by the lungs alone and when by the skin alone" Home inoculated three other children with blood or nasal discharges from measles patients. None of these three subsequently came down with the disease.

A number of subsequent workers failed to confirm his observations with measles blood while a few reported success. Prophylactic inoculation was never exploited on a large scale presumably because of these negative trials and because of the difficulty of obtaining material for inoculation at a convenient time. Difficulty of preserving the infectivity of the inoculum also probably spoke against its adoption. Finally Hektoen, in 1905, in a detailed and critical review of Home's results, seemed to give the coup de grace to the claim that he had achieved the experimental transmission of measles.

Hektoen sweepingly concluded that probably not one of Home's cases developed measles as a result of inoculation because the incubation period

fell short of that in the natural disease, being in no case longer than 10 days, and because Home's failed to exclude the possibility of natural exposure prior to inoculation. The force of Hektoen's objection that the incubation period was too short to be compatible with measles is, I believe, largely dissipated by our own observations which I shall subsequently present, observations on the accelerated development of signs of modified measles following administration of an attenuated measles virus. Hektoen's second objection cannot be so readily met since it must be admitted that chance exposure of some of the subjects may have occurred. Indeed, Home himself was quite aware of this possibility. But as he takes pains to point out, there were at least in two instances in which inoculations were made at the end of August when there were no cases of natural measles in the community. Furthermore, it would seem unlikely that so large a proportion of the children would have developed measles after approximately the same interval following inoculation had previous exposure been responsible.

I suggest, therefore, that the laurels, of which Home seems to have been unjustly denied, should be, in the light of recent findings, restored to this pioneer in the experimental study of measles. In any event, recent investigations which I shall summarize will serve to show that his basic ideas concerning prophylaxis in this disease still live on.

THE VIRUS OF MEASLES

Historical. Any attempt to develop a safe and effective vaccine against an infectious disease must be preceded by identification of the etiologic agent, its isolation in a pure condition or in a state free of other pathogenic microorganisms, its reproduction in sufficient quantities, and its preservation in an antigenically stable form. To those must be added objective tests for resistance of susceptibility and methods for assay of antigenic potency of the vaccine. Home's attempt to inoculate against measles proved abortive because in his time none of these technical requirements could be adequately met. Indeed in the case of measles virus it is only within the past seven years that reliable and effective procedures for meeting them have been developed.

This tardiness is not attributable to lack of effort, since during the preceding fifty years of this century numerous investigators sought to discover susceptible animal hosts, and to propagate the virus in tissue cultures or in the developing chick embryo. The results of these numerous investigations were relatively meager, but certain valuable contributions were made. Indian monkeys inoculated with materials from measles patients

were found by Goldberger and Anderson to develop a mild form of the disease. This observation was confirmed, although certain investigators noted that frequently individual monkeys failed to develop overt signs of infection. This variation in susceptibility rendered the experimental use of these animals difficult or unsatisfactory. Numerous accounts of successful inoculation of other species, such as guinea pigs, rabbits and mice, appeared from time to time but none received general confirmation. Similarly several authors described the propagation of the virus in chick embryos and in chick cell tissue cultures. But again consistent confirmation was lacking. Perhaps the most notable of these studies in which chick embryos were used are those of Rake, Shaffer and Stokes, who in about 1940 inoculated monkeys and a large group of children with materials from serial egg passages that were initiated with infected blood or throat washings from measles patients. Signs of modified disease were observed in a small proportion of the children, but on subsequent exposure to measles little or no protection was afforded and the investigations were not extended. Rake and his co-workers were handicapped by the variable response of the monkey, and lack of any means for the detection and measurement of specific measles antibody whereby they might determine the antigenic potency of the vaccine or distinguish between susceptible and resistant animals or human beings.

Because of these many equivocal results it became increasingly apparent that to achieve further progress two things were needed: first, a simple culture system which would be affected by the virus in such a way as to make it easily apparent that viral multiplication had occurred within the system itself; second, experimental animals of uniform susceptibility to the virus. Given these two essential tools, one felt confident that reliable techniques could be readily developed for isolating the virus, studying changes in its virulence, and producing it in quantity. Procedures would also be provided for the detection and assay of antibodies and for determining the potency of any vaccines that might be developed.

Isolation in Tissue Culture. It seemed that the tissue culture techniques which Weller, Robbins, and I had successfully used in the propagation of the polioviruses might at least provide us with one of these tools. Accordingly, in 1954, with Dr. Thomas Peebles, blood and throat washings taken in the early exanthematous phase from typical cases of measles were added to roller tube cultures of normal human kidney tissue. After a considerably longer incubation period than is characteristic of poliovirus

we saw changes in the new outgrowth of renal epithelial cells which were quite unfamiliar and therefore exciting.

These changes occurred focally and consisted of loss of cell boundaries with consequent mingling of cytoplasm to form an extensive mass in which numerous nuclei lay embedded. When stained, affected areas closely resembled syncytia or multi-nucleated giant cells. In nearly every nucleus within the syncytium large, intensely staining eosinophilic inclusion bodies were seen, surrounded by a clear halo. In the syncytial cytoplasm eosinophilic bodies of irregular size and shape were frequent, but not invariable, features. These cytopathic effects proved to be reproducible in series when the fluid from an infected culture was transferred to a fresh preparation. The concentration in the culture fluid of the transmissible agent thus revealed could now be easily determined by testing increasing dilutions of the fluid for their capacity to induce these changes. Filtration through bacteria-retaining membranes and failure to demonstrate bacteria in infected fluids served to provide definite evidence for the viral nature of the agent we had cultivated. These observations have been repeatedly confirmed by other workers.

Serological Identification. Evidence strongly suggesting that this agent was in fact the virus of measles was obtained by showing that the distinctive cytopathic changes failed to appear when the serum from patients convalescent from measles was mixed with the virus-containing tissue culture fluid and introduced into fresh cultures. In contrast sera taken in the early acute phase exhibited no such suppressive or neutralizing effect. The virus-neutralizing effect of convalescent phase sera was thus shown to depend on a specific antibody that develops during the course of measles. Furthermore, we found that fluids from infected cultures contained an antigen which fixed complement with convalescent but not with acute phase measles serum. Very recently Chany in France and Rosen in the United States have shown that measles virus hemagglutinates rhesus monkey red cells and that this hemagglutination is specifically inhibited by convalescent measles serum.

Effect of Virus on Monkeys. Additional assurance that we were dealing with the virus of measles was obtained when we inoculated fluids from infected cultures into susceptible monkeys. Before these inoculations were done, since we now had the techniques available, and since we had in mind the irregular response of these animals noted by earlier workers, we tested the sera of rhesus and cynomolgous monkeys for the presence of measles antibodies. The animals first selected had been held for considerable periods of time in three different laboratories. Virus neutralizing and complement fixing antibodies were found in all eight of the cynomolgous

and in thirteen of sixteen of the rhesus monkeys tested. Frequently the concentration of antibody was high. When a few of these monkeys were inoculated with the tissue culture virus they failed to present any signs of infection. Here apparently we had a satisfactory explanation for the variation in susceptibility of individual monkeys.

To explain these observations it occurred to us that animals with antibodies might have become infected with measles virus or a closely related agent following their capture and confinement in the laboratory. To test this hypothesis we procured, with the assistance of the United States Armed Forces, a number of cynomolgous monkeys that had just previously been captured in the Philippines. No measles antibodies were detected among the first ten animals examined. Subsequently we tested approximately fifty additional cynomolgous monkeys that were shipped to us promptly after capture in the Philippines or Malaya and found them to be quite free of antibody. Comparable results have now been obtained by several other workers.

When monkeys exhibiting negative complement fixation or neutralization tests were inoculated with culture fluids containing the agent one or more manifestations of infection became apparent. These consisted of viremia discernible about the 4th or 5th day which persisted for several days thereafter; moderate leucopenia lasting from about the 7th to the 11th day and a macular measles-like rash on face, trunk, and abdomen. In all animals antibodies specific for the virus began to appear in the blood about the 15th day. Of the various signs, viremia and the emergence of antibody proved to be the most constant and reliable.

Taken as a whole these observations provided the basis for reinvestigation of various aspects of the disease and its causative agent including the problem of specific prophylaxis.

Other Relevant Properties. i) Antigenicity. Since these tools became available a great deal has been learned through the efforts of a number of investigators in Europe, Japan, and America about the nature and properties of measles virus. As background for the studies on vaccination to be reviewed, I shall summarize rapidly a relevant portion of this new information.

Perhaps most important is the demonstration of the high antigenic potency of the virus when it is introduced into the body in an infective state. Serological analyses carried out with the new techniques by Black have demonstrated the presence of neutralizing and complement fixing antibodies in the serum of over 90 per cent of older persons in an urban population in Connecticut. Titers of these antibodies ranged from about

1:16 to 1:64 or higher in more than one half of Black's subjects. These concentrations are not greatly inferior to those attained soon after an attack of measles. The antigenic efficiency of live virus is also emphasized by the rapid mobilization of antibody following infection. Bech, for example, in a recent analysis of a large number of sera from cases in a measles outbreak that occurred in Greenland detected antibodies in a significant proportion as early as 48 hours after onset of rash and in all sera examined within 5 days thereafter. In a subsequent study Bech found that in 90 per cent of persons examined little change in antibody concentration occurred between the 1st and 5th years after an attack.

These observations testifying to the solid, lasting immunity resulting from natural infection encourage the belief that a vaccine composed of an attenuated live virus might likewise induce a satisfactory state of resistance.

Concerning the antigenicity of measles virus after inactivation by heat or chemicals there are so far few published data. However, these are sufficient to indicate that after inactivation by formaldehyde, or by gentle heating, antigenicity may be retained despite repeated injections of the inactivated material which are required to achieve antibody levels approaching those established by infection. Very little is known as yet about the persistence of antibody after immunization with inactivated virus or about its capacity to protect upon exposure to measles.

ii) *Antigenic Homogeneity.* Fortunately for those contemplating vaccination against this disease, it now appears that all strains of the virus are probably antigenically homogeneous. No differences in specificity have been distinguished between viruses isolated in England, Denmark, Japan, and the United States. Moreover, several strains recovered in successive years from cases in the area of Boston, Massachusetts have, in our hands, proved indistinguishable in respect to antigenic composition. It is, of course, possible that strains may be discovered that are not antigenically homogeneous with those known at present. To me, however, the existence of such heterologous strains is unlikely, since one would expect the incidence of second attacks of measles would be higher if different antigenic types existed in nature. This reasoning is supported by the situation in the case of mumps where second attacks are also a rarity and where over a period of twenty years no significant antigenic differences have been noted between the numerous strains of virus that have been studied.

iii) *Capacity to Multiply in Cells of Various Species and Types.* It has been shown in many recent experiments that measles virus, once it has been isolated and grown for a number of generations in human or monkey cell cultures, can be adapted more or less readily to other cell systems such as bovine kidney cells and a variety of continuous or laboratory-maintained lines of human and monkey cells. The Edmonston strain has also been adapted to chick embryo cells. Adaptation to chick cells is of much significance for the problem of vaccination and I shall refer to it again in more detail. Certain of these systems offer advantages over others for particular purposes. Because of the known malignant character of some and the possible malignant character of others it has not been proposed to use any of the available continuous cell lines for propagation of virus to be used in the preparation of vaccines. For this purpose only chick embryo, primary human amnion, and canine renal cell cultures have so far been given serious consideration.

iv) *Stability.* In relation to vaccination with an attenuated virus such as we have developed, the stability of the agent on storage under various conditions is obviously of much practical importance. It has been determined that in complex fluid media such as are used to nourish tissue cultures measles virus rapidly loses its infectivity at 37°C. Happily, at icebox temperatures activity is preserved for several weeks and, as we found in our laboratory, at the temperature provided by solid CO_2, it may be preserved at least for four years. It has also been demonstrated in the laboratories of several pharmaceutical manufacturers in the United States that when suspended in a suitable chemical milieu the virus can be dried from the frozen state with only moderate loss of infectivity. When once frozen and dried, it may be kept in the icebox for many months with little or no decrease in activity. Thus the stability of the measles virus, although far less than that of certain others such as poliovirus, is, under appropriate conditions, sufficient to permit manufacture and distribution of an attenuated virus vaccine.

v) *Yield of Virus in Tissue Culture.* In the future if immunization against measles should be practiced as routine, large quantities of vaccine would be required. Therefore, the characteristics of the growth of measles virus in various cell systems also becomes a matter of practical interest. As I have already remarked, this agent grows at a slower rate in comparison with certain other viruses. In unpublished experiments done several years ago employing human renal cell cultures, we found little evidence of virus multiplication as judged by assay of the nutrient fluid until about the 6th day. Maximum yields were not obtained until about the 9th day.

In chick cell cultures multiplication of the attenuated variant that we have tested as vaccine follows an essentially similar pattern.

The concentration of virus attained in the fluid phase does not usually exceed 100,000 tissue culture infective doses per ml. The quantity of virus within the cells is about 10 times greater. Once viral reproduction has reached a maximum it remains at a steady rate for days or even weeks in primary cell cultures. At this maximum the rate of reproduction is exceedingly rapid as determined by Black and by ourselves in unpublished experiments. This fact gives ample assurance that measles virus can be easily and economically produced in large quantities, since successive harvests of infected fluids can be made from a single set of cultures.

vi) *Cross Reactions with CDV and Rinderpest Viruses.* My final remarks on the properties of measles virus concern the evidence for antigenic relationships between this agent and two viruses causing disease in lower animals, namely canine distemper and rinderpest virus. As long ago as 1928 Bryan postulated, on the ground of pathological and clinical similarities, a relationship between the agents of measles and distemper. Pinkerton and Adams in the United States subsequently embraced this hypothesis and supported it with additional data of the same sort. Karzon in 1955 showed that a large proportion of adolescents have neutralizing antibodies for canine distemper virus. The possibility was then raised that these antibodies develop as a result of infection with measles virus. Subsequently several workers have noted an increase in distemper antibodies following infection with measles virus. In contrast, it has proved impossible to demonstrate in human subjects an increase in antibodies to measles virus following the inoculation of the distemper agent.

Contemporaneously with this work on antigenic crossing between measles and distemper viruses in the United States, Polding and his associates in East Africa discovered that canine distemper and rinderpest viruses were also related. Later Plowright, also in East Africa, revealed a relationship between the rinderpest and measles virus when he found that in children, following an attack of measles, antibodies reacting with rinderpest virus developed in a high proportion of cases.

From the practical standpoint these biologically interesting relationships have stimulated investigation of the possibility of using distemper virus as an immunizing agent against measles. Adams in 1959 reported that the attack rate in an institutional population previously given live distemper virus vaccine was somewhat lower than it was in unvaccinated control groups. Hoenkenga and his associates in a trial carried out in Central America observed a slight reduction in the incidence of measles

in subjects who had received attenuated distemper virus vaccine but concluded that the potency of the distemper virus vaccine was too low to be useful. The use of canine distemper virus as a prophylactic against measles, therefore, does not at the moment appear promising. Further investigation, however, will be necessary before its consideration can be finally disregarded.

VACCINATION AGAINST MEASLES

Attenuation of the Virus. The newer knowledge of the measles virus, as I previously remarked, has been presented essentially by way of background for the trials of the attenuated virus vaccine in man which I shall now consider. These trials were carried out by us in collaboration with a number of investigators, or by others working independently with vaccines prepared in this laboratory from a virus attenuated in its virulence for monkeys.

This virus represents the distant progeny of a strain isolated from the blood of a patient and designated the "Edmonston" strain. When first recovered in human renal cell cultures, it failed to multiply in chick embryos but produced all the signs of measles in monkeys that I have described. After 24 passages in human renal cells followed by 28 passages in human amnion cell cultures, Milovanović of Yugoslavia, who was then working in our laboratory, showed that the Edmonston strain had gained the capacity to grow in the developing hen's egg. After 6 serial passages in the egg, Katz and his associates found, as might be expected, that this agent could be easily propagated in cultures of trypsinized chick embryo cells. During the first four passages in this system the virus exhibited no recognizable cytopathogenic properties, but subsequently caused cell-rounding, formation of small giant cells, and a peculiar morphological transfiguration that we called "spindle cell" formation. This latest effect had been noted early in human amnion cell passages and consists of an elongation of the typical epithelial cell into a shape reminiscent of the fibroblast.

When this chick cell-adapted virus was inoculated by the combined nasal and intravenous routes into susceptible monkeys no overt signs of infection were noted with the exception of a slight leucopenia. In systematic attempts to recover virus from the throat and blood of the inoculated animals none was demonstrated except in one monkey in which virus was detected in a single blood specimen. Obviously the reactions of the monkey to this chick adapted virus were in marked contrast to those induced by the agent when first isolated in human kidney cells. In spite of this absence of signs of infection, all monkeys that received it de-

veloped, by the 21st day, complement fixing and neutralizing antibodies in concentrations comparable to animals given the virus in its original virulent form. When challenged with virulent virus all of the vaccinated animals proved resistant as indicated by failure to exhibit any clinical signs of infection or demonstrable virus in the blood. These results indicated clearly that at some time during its prolonged sojourn *in vitro,* its virulence for the monkey had markedly decreased while the antigenic capacity was fully retained.

A pronounced difference between the capacity of the chick-adapted variant and its progenitor to persist and multiply in the central nervous system of the monkey was also demonstrated. In comparative experiments monkeys were inoculated with one or the other of these viruses into the hypothalamic region and into the cisterna magna. Thereafter specimens of spinal fluid, blood and throat swabbings were collected at frequent intervals and examined for the presence of the respective agent. Tests for antibodies were made at appropriate times. In the animals that received chick cell-adapted virus the agent failed to appear in the spinal fluid, nor was it demonstrated at any time in throat swabbings or blood. In contrast the parent virus was repeatedly recovered from the spinal fluid as well as other materials examined. It is particularly to be noted that antibody responses were comparable in both animals. Histological examination of sections of cord and brain taken 21 days after inoculation revealed no lesions attributable to either the virulent or attenuated agent.

First Trial of Vaccine. Because of its loss of pathogenicity for monkeys with retention of immunogenic properties we decided to explore the possibility of using in man the attenuated virus as a vaccine. We were reassured by these findings indicating that the capacity of the attenuated agent to multiply in the central nervous system of the monkey was clearly less than that of the virulent parent strain, since it would be *a priori* less likely to cause encephalitis in human beings.

Accordingly, we proceeded to prepare with the attenuated agent a vaccine to be used in an initial small trial in susceptible children. Attenuated virus in the 14th chick cell passage was grown in chick cell cultures nourished with the protein-free medium ♯199 of Morgan and Parker. To infected fluid taken from cultures during the period of maximal virus production a small amount of purified human serum albumin was added to stabilize viral infectivity. This material represented the vaccine which was preserved until use in the frozen state. After performance of the usual tests for bacterial contamination and animal toxicity, my associate, Dr. Samuel L. Katz, in October of 1958 administered the vaccine, with the

consent of parents or guardians, to 11 mentally defective institutionalized children who were regarded as susceptible to measles. Criteria for susceptibility consisted of negative history for measles and negative tests for neutralizing and complement fixing antibodies. Approximately 60 tissue culture infecting doses of virus were injected into each child by the subcutaneous route. As controls two susceptible children, who continued to remain intimately associated with the vaccinated group, were at the same time given sterile tissue culture fluid.

The clinical and serological events that followed in the vaccinated children may be summarized. No immediate reactions either at the site of inoculation or of a generalized sort were noted. After six — nine days, a rise in rectal temperature was recorded in eight of the vaccinated children which lasted from 2 to 5 days with a mean duration of 3.5 days. Maximal temperature recordings ranged from 100.8 to 103.8 F. with a mean of 102.8 F. After defervescence a pink, macular, nonpruritic, discrete rash af varying extent appeared in nine children. In five of them it might easily have escaped superficial examination. Koplik spots were seen in one child with a questionable rash. The rash appeared usually about the 11th day and lasted from 1 to 3 days. In four children it was seen only about the face and neck, in four others in addition to these areas the trunk was involved. In general the picture and of a mild, much modified measles. The most striking feature was the absence of any sign of disability. Notable, too, was the absence of any signs of involvement of the respiratory tract. In every case the child went about his usual routine with no loss of appetite or impairment of excretory functions. Two of the vaccinated children and the two control children exhibited no clinical signs of infection. Specimens of blood and throat washings obtained from each of the thirteen children on the 5th, 9th, and 15th day after vaccination were tested in tissue cultures for the presence of measles virus. None was demonstrated.

By testing specimens of serum taken from each child at appropriate intervals, emergence and decline of complement fixing and neutralizing antibodies were followed. Antibodies developed in all of the vaccinated children. Complement fixing antibodies were first detected on the 15th post vaccination day and attained the highest concentration recorded on the 22nd day. At the maximum, serum dilution titers ranged from 32 — 256. This range, as I shall again emphasize, approaches that exhibited by most individuals convalescent from natural measles. On the 36th day titers of neutralizing antibodies ranged from 90 — 300, again a range comparable to that most often encountered in early convalescence. At intervals

during the 2½ years elapsing since vaccination antibody levels have been determined. A curve of the geometric mean titers at these intervals is shown in Figure 1. It will be seen that from the maximum neutralizing antibodies declined by about a factor of 4 by the end of one year. Since that time, however, the level has remained essentially unchanged. The initial decline in complement fixing antibody was more pronounced and the titers finally attained were lower.

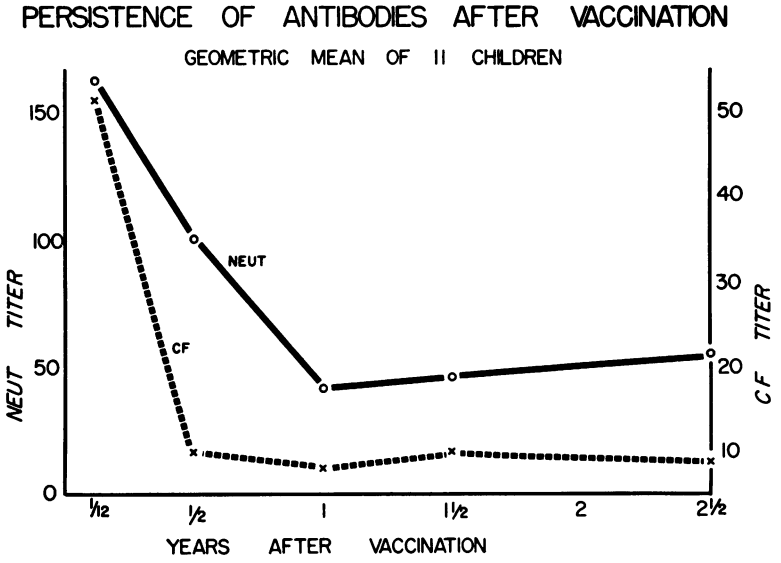

FIG. 1.

During this observation period of 2½ years, cases of natural measles have not occurred in the institution. We may confidently assume, therefore, that the vaccinated children have not been exposed. This assumption is also supported by the fact that tests for measles antibodies in the sera of the two control children have been consistently negative. Accordingly, the appearance and persistence of antibodies may be attributed to the effect of the vaccine.

From the results of this first small trial it was also concluded that:

i) The virus employed as vaccine is to be identified as the agent of measles.

ii) Under the conditions to which it was subjected in the laboratory, its virulence for man as well as for the monkey was reduced, but to a less degree.

iii) The virus is not transmitted from vaccinated subjects to susceptible contacts.

Additional Studies of the Original Vaccine. Because of the elevated temperatures noted in a few of the children in this first group an attempt was made to induce further attenuation of the virus. Accordingly, the vaccine strain was subjected to six additional chick embryo and four chick cell passages. A vaccine was prepared with virus from the last passage and, to distinguish it from the original vaccine or Vaccine A, it was referred to as Vaccine B. When Vaccine B was tested in susceptible children by Lepow and her associates in Cleveland, Ohio, they obtained the same spectrum of reactions which we had observed with Vaccine A.

Approximately 470 children and adolescents have now received parenterally either vaccine A or B. Since no difference in the effects of these vaccines was noted, we have assumed that all these subjects received comparable inocula. This large group of 470 children was made up of a number of small groups consisting of patients in institutions for the mentally retarded, patients attending a neurological clinic, patients with cystic fibrosis of the pancreas, and normal children in institutional schools and in the home. A number of investigators collaborated in these studies and have published or will soon publish their observations. We are greatly indebted to them for their interest and skillful management of these trials.

The relevant serological and clinical findings in the first 272 children in the series may be summarized as follows. To be first noted is the fact that many of the children did not respond to vaccination by the development of antibodies. Failure to respond was correlated in 101 of 107 cases with the presence of measles antibody in the pre-vaccination serum specimen suggesting that most of these non-reactors had previously experienced an infection. Of the 171 children in whose pre-vaccination sera no antibody was demonstrated, 165 or 96.5 per cent developed antibodies in significant concentrations following vaccination. We cannot clearly account for the failure of six initially negative reactors to respond. Possibly the quantity of virus in the inoculum was insufficient, but, more probably, pre-existing undetected low levels of antibody were responsible.

However this may be, it was evident that the incidence of serological response to vaccination among the susceptibles was gratifyingly high. As shown in the examples listed in Table 1, antibody concentrations attained after vaccination, approached those characteristic of convalescent phase sera from measles patients. The differences observed between the

mean antibody titers of the various groups probably depend in large part on technical factors.

The principal clinical reactions to vaccination among the children with initial negative serologic tests consisted of fever and exanthem. Fever usually began on the 7th or 8th day and lasted on the average about 3 days. The average maximal temperature by rectum was 102.4°F. The upper limit of 106°F. occurred in a single child who exhibited no other signs of illness and whose temperature quickly dropped after aspirin

TABLE 1. COMPARISON GEOMETRIC MEAN TITERS OF ANTIBODY AFTER NATURAL MEASLES AND MEASLES VACCINATION

Author	*Subjects*	*Stimulus*	*No. sera*	*Geo. M. Tit.**	*Range Tit.**
Bech[2]	Normal	Natural Measles	72	195	32-1024
Haggerty[9]	Normal	Vaccine A	32	121	16-256
Katz and Enders[9]	Mentally Retarded	Vaccine A	11	70	32-256
Black[9]	Mentally Retarded	Vaccine A	9	75	16-256
Krugman[9]	Mentally Retarded	Vaccine B	17	206	64-1024

* Reciprocal serum dilution end-point.

was given. In twenty-three or 14% of 165 children who developed antibodies no significant febrile response was recorded.

Only about one-half of the subjects developed a rash — definitely fewer than in our initial small trial. Again I would stress the fact that the rash usually appeared on the 10th to the 11th day when the temperature in most cases had returned to normal. Thus the interval between inoculation and appearance of rash corresponded with that noted by Francis Home. In appearance and distribution the rash was reminiscent of that associated with measles modified by gamma globulin. Koplik spots were seen only in a small proportion of the children. With very few exceptions the absence of disability was remarkable.

The results in two other groups who have received vaccine of the same manufacture deserve special comment. One consisted of children with cystic fibrosis who attended the clinic of Dr. Harry Schwachman at the Children's Hospital Medical Center, Boston, Massachusetts; the other of children suffering from a variety of neurological and metabolic disorders who attended the clinic of Dr. Frederic Gibbs and Dr. Ira Rosenthal of the Department of Pediatrics, University of Illinois College of Medicine, Chicago, Illinois.

The children with cystic fibrosis were chosen for vaccination because in such patients natural measles is often followed by serious disease of the lower respiratory tract which is frequently the seat of prior infection by bacteria or fungi. In Dr. Shwachman's clinic, as elsewhere in the United States, it is the practice in these patients to administer gamma globulin as a prophylactic measure after each exposure or presumptive exposure to measles, a troublesome and not always reliable procedure.

TABLE 2. CLINICAL REACTIONS AFTER MEASLES VACCINATION IN 125 PATIENTS WITH CYSTIC FIBROSIS

	No. of patients and incidence			
	Uncorrected		*Corrected*	
Reaction	*No.*	*%*	*No.*	*%*
None	51	41	26	26
Fever	37	30	37	37
Rash	5	4	5	5
Rash—Fever	32	26	32	32

An effective means of inducing active immunity, therefore, would be especially desirable under these conditions.

Measles vaccine has now been given to 125 patients with cystic fibrosis. Incidence of reactions to vaccination, as summarized in Table 2, was apparently somewhat less in this group as compared with that in the other children we have discussed. The difference, however, is probably to be attributed largely to the inclusion in the cystic fibrosis group of a number of initially immune children. Thus antibody determinations carried out on the pre-vaccination sera of 61 of these children indicated that about 20% were initially immune. Using this factor to correct the data for the entire group it is evident, as shown in the last column of Table 2, that the incidence of rash and fever approached that previously observed. Post-vaccination antibody titers in a sampling of 48 of the patients with cystic fibrosis who responded serologically to vaccination ranged from 1:8 to 1:512 (dilution of serum) or higher. These results suggest no impairment of the antibody-forming mechanism in patients with this disease. Unusually severe or abnormal reactions were not observed nor was the underlying illness affected in any way. It would seem, then, that individuals with this serious and debilitating illness tolerate the vaccine well.

A group of children were studied by Dr. Gibbs and Dr. Rosenthal with a special purpose in view: namely, to determine whether after

vaccination abnormalities in the electroencephalogram might occur. Previously Gibbs and his co-workers had found in a series of over 600 cases of natural measles accompanied by no clinical evidence of encephalitis that 51 per cent exhibited abnormal slowing of the brain waves. It was shown that these abnormal patterns were not due to fever *per se*. Gibbs and Rosenthal have now completed electroencephalograms in 40 children taken just before and 10 days after measles vaccination. Twenty-eight of the subjects were considered to be susceptible on the basis of serological tests. The reactions to the vaccine were entirely comparable to those I have already described. With a single exception no deviations from the prevaccination electroencephalographic patterns were noted. The exceptional child was suffering from a febrile upper respiratory tract infection at the time the second electroencephalogram was made. It has been stated by Gibbs that such infections may be accompanied by the type of transitory slowing that was recorded in this case.

These essentially negative electroencephalographic findings following vaccination led Gibbs and Rosenthal to conclude that fear of abnormalities in the electroencephalogram similar to those induced by natural measles can be excluded as a possible contra-indication to large scale use of the vaccine.

Prophylactic Effect of Vaccination. We may now ask how effective this vaccine has proved to be in the prevention of illness following exposure to measles. So far as we are aware no case of the disease has as yet developed in any of the approximately 500 children that have been vaccinated. Instances of known exposure, however, have not been numerous. Even so, there is available sufficient evidence to show that protection conferred by vaccination is of a high order. Thus, Krugman working in an institution for mental illness reported that none of 23 vaccinated children developed measles after intimate and prolonged exposure. In the same ward, 17 among 23 unvaccinated children came down with the disease and, in an adjoining ward, 29 among 50 unvaccinated inmates acquired measles. In Krugman's study the vaccine was administered about seven weeks before exposure occurred. Of the 23 vaccinated children who subsequently proved resistant on exposure, 18 children had exhibited the usual clinical and serological responses to vaccination.

Complete protection has also been observed by Lepow, Haggerty, and Schwachman among 35 vaccinated children considered to be susceptible at the time of vaccination. These children were exposed in the home to siblings with measles. The interval between vaccination and exposure in these family studies varied from 2 months to 16 months.

That the measles attack rate ranges from 75 to 95 per cent following exposure is known to everyone. Accordingly, these observations while limited seem sufficient to justify the conclusion that the attenuated virus vaccine prepared and administered in the manner I have described is a highly efficient prophylactic during relatively short periods. Only with the passage of time can the duration of protection be finally assessed. Nevertheless, the persistence of neutralizing antibodies for at last 2½ years as demonstrated in our first trial encourages us to believe that immunity may be durable, possibly as durable as that established by the natural disease.

Trials with Other Vaccines. Recently a number of other workers have carried out trials with living measles virus vaccines. Some of these have appeared in the literature; others are still in progress. Certain of the vaccines employed were prepared with our strain of chick-adapted virus but subjected to different conditions of cultivation. Others such as those tested by Smorodintsov in Leningrad consisted of entirely different strains. It is not my purpose to review these findings in detail. In general it can be said that with chick-adapted virus the characteristics of the clinical response have been broadly comparable to those I have described. Certain differences, however, were noted. For example, Schwarz and his co-workers isolated virus from the blood of one of two vaccinated children and Smorodintsev described frequent viral recovery from blood and throat washings from his subjects. McCrumb in Baltimore who studied a vaccine consisting of Edmonston chick-adapted virus that had been propagated for a few passages in canine renal cells, also encountered the agent more often in blood and throat washings. Hoenkenga and his associates employing chick cell vaccine reported that four poorly nourished children were sufficiently ill to be cared for in hospital during short periods. Satisfactory antibody responses were obtained by all these workers and several also reported a high level of resistance as measured by exposure to measles.

FUTURE DEVELOPMENTS

In this paper, I have attempted to outline some of the salient features of the history and present status of vaccination against measles. In conclusion we may consider very briefly what the future of vaccination may be. First it seems clear that the need for a simple, safe, and effective method for promoting lasting immunity will ensure continuation of efforts to perfect the vaccines now at hand. The need is emphasized merely by citing the incidence of measles in a country like the United States where

the annual reported attack rate outdistances that of any other notifiable disease. For example, about 5½ million cases were notified during the 10 year period from 1950 to 1959. The total incidence during this time is conservatively estimated at about 22 million cases. One has only to visualize the vast amount of discomfort hidden behind these figures as well as the additional burdens placed upon parents and the loss of time for all concerned, to be convinced that elimination of measles would be desirable.

When we add to such considerations the danger of serious and often fatal complications such as encephalitis, the need for a good prophylactic receives additional emphasis. To support this statement I may note that in the United States reported deaths from measles during the 3 year period from 1956 to 1959 totaled 1471, whereas deaths from poliomyelitis were recorded as 1042. Measles exceeded poliomyelitis mortality by about ⅓ during this period. Because measles encephalitis is known to vary with time and place, it is difficult to estimate accurately the incidence of this most serious complication. However, on what appears to be a fair assumption, encephalitis develops in about one out of 800 patients. On this basis I have calculated that about 28,000 cases occurred in the United States during the ten year period from 1950-1959.

In countries where nutrition and general sanitation are defective the frequency of pneumonia and other complications may be much higher with a corresponding increase in mortality. Under these conditions vaccination would seem to offer the only means of control that will be available for some time to come.

Because of the occasional high fever following vaccination, the discovery of means whereby such reactions may be eliminated will be a major objective of future research. Already trials are in progress in which modifying doses of gamma globulin are administered simultaneously with chick attenuated virus vaccine. Initiated by McCrumb, these trials are being extended by Krugman, Stokes, and Hilleman as well as others in the United States. It is even now apparent that with this procedure post-vaccinal reactions are considerably reduced and it has been shown that antibody formation is not usually suppressed although it may be somewhat diminished. Little or nothing is yet known regarding the degree or duration of the resistance that may be established by this technique. If found effective, sero-vaccination of this sort might be adopted in certain countries; but in others, it may be regarded as too complicated and too expensive. Much effort will be exerted, I feel sure, to push further the attenuation of the virus itself.

Finally, trials of the presently available chick cell adapted vaccine should be extended until they include sufficient numbers to give complete assurance that it is never encephalitogenic. Once its innocuity in this respect is demonstrated and its reactivity somewhat reduced we may predict that Home's ancient dream will at last become a reality.

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