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LIVING MODIFIED VIRUSES AS IMMUNIZING AGENTS*

BY

HERALD R. COX, Sc.D.

Johns Hopkins University, Baltimore, Maryland

*(From Viral and Rickettsial Research, Lederle Laboratories Division,
American Cyanamid Company, Pearl River, New York)*

Every medical investigator of the twentieth century counts among his scientific forebears the two great Germans Paul Ehrlich and Emil von Behring. Born a day apart, friends and co-workers, both Nobel Prize winners, appropriately they have been called "twin sons of science and humanity." I leave to others the enumeration of their many accomplishments.

One writer has said that Behring forged the sword of serotherapy, but Ehrlich ground its edge. On this occasion, no one can speak of the weapons against disease we are now creating without first acknowledging the power of those fashioned by Ehrlich and von Behring.

Jenner and Pasteur

The purpose of this paper is to review a mighty weapon we now possess—namely, living modified viruses as immunizing vaccines. Unquestionably, Jenner's use of cowpox or vaccinia virus on a large scale to protect against smallpox was the first instance of immunization with a living, attenuated virus. It had been recognized for many years in England, France, and Germany that milkmaids who had contracted cowpox were spared during ravaging epidemics of smallpox. The observation of this fact was not original with Jenner. The merit of his work rests upon the fact that he started out with the hope of making his conception a permanent working principle in science, based upon experimental demonstration, and he succeeded.¹ In short, Jenner transformed a rural tradition into a living prophylactic principle. Popular aversion to vaccination was overcome, and the practice spread rapidly in Europe and America. The effect of Jenner's work is apparent in the rapid advances serotherapy and immunology have made, and in the results of compulsory vaccination in Germany and Holland, where the mortality curve of smallpox approaches zero. Striking, indeed, was the relative immunity of the German army during the Franco-Prussian War in 1870-1, in which the unvaccinated French prisoners lost 1,963 out of 14,178 cases of smallpox, whereas the Germans, who had been revaccinated within two years' time, had 4,835 cases with only 278 deaths.²

We are indebted to Pasteur, who, in his studies on fowl cholera, first recognized the importance of microbic variation for practical purposes of medical use.³ His

discovery of preventive inoculation with attenuated living agents resulted from an accident. During a vacation period, virulent cultures of the chicken cholera organism left in the laboratory became avirulent, but were found to act as preventive vaccines when inoculated birds were later challenged by injections of living, fully virulent material. The attenuated culture could be passed through several successive generations and still retain its immunizing property. Pasteur later studied the agents of anthrax, swine measles (*rouget des porcs*), and rabies, and established the important principle that the invasive and pathogenic properties of a virus can be altered by successive passages through appropriate hosts. This work gave rise to one of the most illuminating ideas in the history of medical science: that the origin or extinction of an infectious disease may be simply the result of the enhancing or attenuation of its virus by external conditions. Pasteur applied the principle of passing the virus serially in an unnatural host to develop a rabbit-adapted, fixed rabies virus which was used as a living vaccine.

Viral Agents

The basic investigations of Jenner and Pasteur have been gradually extended during the intervening years so that to-day there are at least 13 different viral agents being used as living attenuated vaccines in the practice of human or veterinary medicine. These are: African horse-sickness, blue-tongue, canine distemper, fowl-pox, hog cholera, infectious bronchitis, laryngotracheitis, Newcastle disease, pigeon-pox, rabies, rinderpest, vaccinia, and yellow fever.⁴ Three of these—rabies, vaccinia, and yellow fever—are used extensively for the immunization of man. The remainder, plus rabies, are used widely in the field of veterinary medicine. It is interesting to note that fowl-pox, infectious bronchitis, laryngotracheitis, Newcastle disease, pigeon-pox, and vaccinia are viral strains of relatively low pathogenicity recovered directly from their natural hosts (Table I).

TABLE I.—*Modified Living Vaccines Derived from Natural Hosts*

Disease	Natural Host	Vaccine Source
Fowl-pox	Chicken	Chick embryo
Infectious bronchitis	"	" "
Laryngotracheitis	"	" "
Newcastle disease	"	" "
Pigeon-pox	Pigeon	"
Smallpox	Man	Calf

*Presented at Frankfurt-on-Main, Germany, March 16, 1954, on the occasion of the 100th anniversary of the birthdays of Paul Ehrlich and Emil von Behring.

Seven of the immunizing agents—African horse-sickness, blue-tongue, canine distemper, hog cholera, rabies, rinderpest, and yellow fever—are laboratory-induced or man-made mutants (Table II).

TABLE II.—*Laboratory-induced Modified Living Vaccines*

Disease	Natural Host	Vaccine Source
African horse-sickness	Horse	Mouse brain
Blue-tongue	Sheep	Chick embryo
Canine distemper	Dog, ferret, mink	" "
Hog cholera	Swine	Rabbit "
Rabies	Man, dog, cattle, etc.	Rabbit, chick embryo
Rinderpest	Cattle, buffalo	Goat, rabbit, chick embryo
Yellow fever	Man	Chick embryo, mouse brain

Apart from the above, there are several other viruses which have been used at one time or another on an experimental scale for the immunization of man or animals.⁴ Some of these are: Colorado tick fever,⁵ dengue fever,⁶ infectious hepatitis,⁷ influenza,^{8, 9, 10} mumps,^{11, 12} poliomyelitis,^{13, 14} and Rift Valley fever.¹⁵

Properties Required of a Good Vaccine

I believe that all those who have had sufficient experience will agree that the greatest success in immunizing against viral infections has been achieved by the use of living mutants lacking the invasiveness and pathogenicity of the parent strain, but retaining the same antigenic structure. In practically all cases, the immunity induced by living attenuated vaccines is of higher degree and longer duration than that produced by inactivated viral preparations. To demonstrate, I mention the successful use of vaccinia virus and yellow-fever vaccine in protecting man; and the durable results obtained with the Flury strain rabies vaccine in dogs; the rabbit-adapted hog cholera virus in swine; and the quadrivalent blue-tongue vaccine in sheep. Each of these preparations has been extensively used and has passed the test of time with flying colours.

Certain specifications must be met in order to secure a practical and reliable vaccine that can be widely used with confidence.

(1) The vaccine—when applied by the proper manner and route—must be safe for general use. It must not induce a severe or fatal infection in the host it is intended to immunize.

(2) The vaccine must be attenuated to such a degree that it will not only be innocuous for the host but will have such low virulence or "spreading" potential that it cannot be transmitted readily from one susceptible host to another, thereby conceivably regaining its original invasiveness and virulence.

(3) The product must be adequately tested and known to be free at all times of any other viral, rickettsial, or bacterial contaminant that could be considered, even remotely, as potentially pathogenic.

(4) The product must contain enough antigen to bring about an adequate immunizing infection under all types of field conditions. We know that to be effective as an antigen an attenuated live virus must multiply in the tissues of the host. For this to take place a sufficient number of living virus particles must be present in the inoculum. The optimal range of virus content must be quantitatively controlled and accurately determined individually for every lot of vaccine produced.

(5) The product must be rigidly controlled not only quantitatively but in a biologically qualitative manner as well, to ensure that the infectious agent has enough invasiveness to establish and guarantee immunity. Thus, the infectious agent must be sufficiently attenuated to render it safe for use, but not over-attenuated to the point that it ceases to be a good immunogenic antigen.

(6) The product must be stable, so that it will retain its immunogenic capacity unimpaired under all types of adverse conditions such as light, moisture, temperature, and travel (shipping conditions) for long periods of time.

(7) The product must be produced by a practical method, so that it will not be too expensive, but can be afforded by all who need it.

The above are the specifications which research scientists and production workers must meet for a marketable vaccine worthy of a good reputation. As might be surmised, there is no ready formula available at present for making viral vaccines by a mould or template system. Every vaccine now available has come about only after many devoted workers have put in long years of painstaking and often heart-breaking toil.

It seems to me of interest to describe briefly a few viral agents commonly employed to-day as living attenuated vaccines.

Yellow Fever

I believe we can safely say that yellow fever vaccine represents the most successful attenuated living viral immunizing agent so far "hand tailored" in the laboratory for use in man. Two strains are used currently for human vaccination: the French neurotropic (mouse brain) and the 17D (chick embryo). Both types of vaccine are based on the original finding of Theiler^{16, 17} that white mice, if inoculated intracerebrally, are susceptible to yellow fever, and that a fixed virus for mice, with a shortened incubation period and heightened virulence, can be produced by repeated passage through these animals. The original French strain of yellow fever virus was isolated from a Syrian in Dakar in 1929¹⁷ and was adapted to mice by Theiler in 1930.¹⁸ It lost its capacity to produce fatal visceral yellow fever in rhesus monkeys. This mouse-brain fixed neurotropic strain of virus has been used on a large scale in the French colonial populations in Africa.^{19, 20} The vaccine is essentially dried infected mouse-brain tissue which is resuspended in a solution of gum arabic and applied to the scarified skin.

Other investigators, largely as a result of animal experiments, considered the French neurotropic vaccine to be too virulent for human use. Consequently, Theiler and his associates carried out a long series of investigations which resulted in the chick-embryo-adapted 17D strain vaccine.²¹ The 17D strain of virus originated from the Asibi strain and was derived by cultures as follows: 18 passages in minced mouse embryo/Tyrodé's solution; 58 generations in whole chick embryo/Tyrodé's solution; and 160 subcultures in chick embryo (head and spinal cord removed)/Tyrodé's solution.

Inoculation of monkeys by neural routes showed that the 17D strain had to a great extent lost its power to produce fatal encephalitis between the 89th and 114th chick-embryo-tissue culture passages.²¹ More extensive experiments in which rhesus monkeys were inoculated intracerebrally with the 17D virus showed that the incidence of fatal infection for this host is from 5 to 10%, and varies with parallel cultures of the strain.²² In addition to losing neurotropism for mice and monkeys, the 17D strain became less viscerotropic for these animals. As a rule, monkeys inoculated with the 17D strain by extraneural routes showed no fever or other signs of illness, and only minimal amounts of virus could be demonstrated in their blood. Such monkeys developed specific antibodies and were solidly resistant to challenge with highly virulent strains of yellow fever. After extensive experimentation in monkeys, the 17D strain was used for human vaccination. With the loss of both neurotropic and viscerotropic properties, as demonstrated in monkeys, this seemed to be the virus strain of choice for human vaccination—a decision which, in the main, has been well justified. It should be mentioned, however, that the reason for the relatively rapid change in virulence of the 17D strain, which occurred between the 89th and 114th tissue culture passages, was and still is completely unknown.

Yellow fever vaccine is now produced from developing chick embryos. The vaccine consists of frozen and dried infected chick-embryo tissue reconstituted in sterile salt solution, and is injected subcutaneously in a 0.5-ml. dose.

Like smallpox, the yellow fever vaccine induces immunity by actually producing an infection in the vaccinated individual. Small quantities of active virus appear capable of producing immunity in a man.²³ The minimal quantity of virus in the finished vaccine has been set at 500 LD₅₀ for white mice inoculated intracerebrally. The mild clinical reaction produced by yellow fever vaccine is rather typical of all living attenuated virus vaccines. Thus approximately 5% of vaccinated persons show, on or about the seventh day, a reaction consisting of malaise, headache, backache, and slight fever. This usually lasts only about 24 hours and is seldom severe enough to interfere with daily routine. Immunity, demonstrated by neutralizing antibody production, is manifest by the seventh to ninth day after vaccination. The titre of antibodies in persons vaccinated with 17D virus is as a rule low, but antibodies may be found by the seventh to ninth day after vaccination and they persist for considerable periods of time. Studies carried out by Dick and Gee²⁴ indicate an immunity persisting for at least nine years. The immune response may be less in children than in adults. Millions have been vaccinated with the 17D vaccine.¹⁷ Perhaps the most conclusive demonstration of the effectiveness of yellow fever vaccination is the fact that since it was introduced no accidental cases of yellow fever have occurred in laboratory workers. Prior to the development of a vaccine such infections were extremely common and several cases were fatal.

The more recent studies of Hahn suggest that cutaneous application may provide a more effective and yet less expensive method of vaccination against this disease.²⁵ Hahn's work indicates that 17D chick-embryo vaccine applied topically in human beings induces a high degree of immunity within 10 days. This vaccine is prepared by grinding the virus-infected chick embryos with gum arabic solution and freeze-drying the mixture, which is rehydrated with saline or distilled water at the time of use. Additional experiments have been made with mixed vaccinia and yellow fever viruses. The combined vaccine has given encouraging results in field trials.

It should be noted that no chemical or physical method has been found for inactivating yellow fever virus—that is, for eliminating its pathogenicity and yet retaining its antigenic properties.²²

Rabies

In an effort to develop a strain of rabies virus that could be used safely for immunization, Pasteur and his associates²⁶ passed the virus intracerebrally in series in a number of animal species. Intracerebral serial passage in rabbits produced a strain of rabies characterized by a very short incubation period. Such modified strains were called fixed, to differentiate them from the natural or street virus strains. After about 100 passages in rabbits the fixed virus had little lethal capacity when injected subcutaneously into dogs. After Pasteur many workers, too numerous to mention here, developed both living and killed types of rabies vaccines for use in various parts of the world. All of these vaccines were prepared from infected central nervous tissues, and suffered from the fact that they often gave rise to neuro-paralytic reactions, as well as offering a relatively short span of immunity.

Seeking to overcome some of the shortcomings of the brain-tissue vaccines, the so-called Flury-strain vaccine was developed in my laboratory. Briefly, it is produced in developing chick embryos from a strain of rabies isolated by Leach and Johnson²⁷ from the brain of a girl named Flury.²⁸ The Flury strain is unique in that it is the only strain of rabies virus, so far as I know, that has been maintained entirely in non-mammalian hosts since its original isolation from human tissues. Johnson carried the strain for 136 serial passages in the brain tissue of 1-day-old baby chicks. He observed that the chick-adapted virus showed a lessened pathogenicity for rabbits, mice, and dogs. He also reported that although rabies could be induced in dogs by intracerebral inoculation of the chick-adapted strain, yet at necropsy no virus could be demonstrated in the salivary

glands or saliva.²⁹ This was a most important observation, since if rabies virus is not present in the saliva of a rabid dog it is most unlikely that the dog could serve as a vector in transmitting the disease by biting other animals. Dr. Johnson very kindly made the 136th chick-brain passage of the Flury strain available to us. Dr. Hilary Koprowski and I succeeded in adapting the strain to grow in developing chick embryos.³⁰ The strain is pantropic for the chick embryo—virus being found in all embryonic tissues, including the blood.³¹ Furthermore, even at low passage levels, the virus was relatively innocuous for guinea-pigs and rabbits tested by peripheral routes.

Following good results obtained in rabbits and guinea-pigs, extensive laboratory tests were carried out in dogs. These may be summarized by stating that dogs which have been inoculated intramuscularly with a single 3-ml. injection of a 33½% chick-embryo suspension show uniform resistance within 18 to 20 days to massive street-virus challenge inoculated bilaterally into the masseter muscles of the jaw. Animals immunized with Flury-strain vaccine resist challenge with street-virus strains not only of American origin, but also with strains isolated in Greece, India, and Central America (vampire bat origin).³² The Flury strain is immunogenic only in its living state. No satisfactory physical or chemical method has been found that will inactivate the virus without destroying its antigenic capacity. Laboratory tests have been confirmed by extensive field tests in Georgia, in the State and City of New York, more recently in Malaya, and in a mass rabies control programme sponsored by the World Health Organization in Israel.³³ At present the Flury rabies vaccine is licensed and recommended for vaccination of dogs only, but impressive results have been obtained in the vaccination of other animals, particularly cats and cattle (cats received a 1.5-ml. dose, and cattle 7.5 or 15 ml.). All evidence to date indicates the superiority of intramuscular vaccination over the subcutaneous route.

Flury Strain Tests

The Flury strain of rabies virus is propagated in the developing chick embryo. The infected chick-embryo-tissue suspension is frozen and dried with stabilizers to preserve maximal viral activity. Each batch of vaccine is tested in three ways: for adequate virus content by intracerebral inoculation of mice; for freedom from other potential pathogens by inoculation into dogs, guinea-pigs, and mice; and for immunogenic capacity by street-virus challenge of vaccinated guinea-pigs. Good vaccines as a rule must contain approximately 10^{-3.5} LD₅₀ as judged by intracerebral titration in mice. More than a million dogs have been vaccinated with the Flury-strain product, and in no instance has the modified virus showed a tendency to regain its original virulence and transmissibility.

After prolonged serial passage of the Flury strain in chick embryos, a marked decrease in its pathogenicity for laboratory animals was observed. Up through the 176th chick-embryo passage, the Flury strain consistently killed intracerebrally injected adult mice, with LD₅₀ titres ranging from 10^{-4.5} to 10^{-4.8} or 10⁻⁴. From the 178th to the 208th egg passages the Flury strain was devoid of lethal activity as measured by intracerebral injection of adult mice. Subsequent studies have revealed that the high passage levels of Flury virus in chick embryos have lost their pathogenicity for young adult mice, but have retained pathogenicity for suckling mice 8 days old or younger. What is more important is that the strain has apparently maintained its immunogenic capacity for dogs, guinea-pigs, and adult mice.

The finding that such a highly neurotropic virus as rabies can become so greatly attenuated in virulence and invasiveness is thought-provoking. The question arises, "Will the high-passage-level Flury virus retain its immunogenic capacity, or will this property be lost on continued passage in chick embryos?" One thing certain, however, is that the Flury strain will never regain its original virulence for dogs or other animals by continued serial passage in chick embryos. As a matter of fact, our problem will be to

preserve the immunogenic capacity of the strain for mammalian hosts. This is something we are fully aware of, and fortunately can control.

The Flury rabies vaccine unquestionably confers a solid degree of protection to vaccinated dogs for two- and three-year periods against a massive challenge dose of street virus, as shown by Tables III and IV.

TABLE III.—Results of Immunity Test in Dogs Vaccinated 24 Months Previously with Flury Rabies Vaccine

Authors	Type Vaccine	Mortality Ratio
U.S. Public Health Service in co-operation with Lederle	Avianized (Flury)	0/33 (0%)
	Lederle Controls (non-vaccinated)	18/33 (54.5%)

TABLE IV.—Results of 39 Months' Immunity Test in Dogs Vaccinated with Different Types of Rabies Vaccine

Authors	Type of Vaccine	Mortality Ratio
U.S. Public Health Service in co-operation with Lederle	Phenolized (commercial)	8/34 (23.5%)
	Ultra-violet (commercial)	7/30 (23.3%)
	Avianized (Flury)	0/30 (0%)
	Lederle Controls (non-vaccinated)	31/36 (86.1%)

Table IV shows a comparative immunity test in dogs vaccinated 39 months previously with Flury living-virus vaccine or with phenolized or ultra-violet-inactivated commercial vaccines prepared from nervous tissues.^{34 35} Frankly, we were surprised that the killed vaccines—the phenolized and ultra-violet preparations—conferred as good protection as the three-year challenge test revealed. However, the Flury living virus-vaccine obviously gave the best performance on challenge.

In additional studies,³⁶ it has been determined that hyper-immune serum may supplement Flury-strain rabies vaccine in cases of extremely severe exposure, and that immune serum does not interfere with or block the immunizing capacity of the living virus agent.

Results of work looking towards the use of the Flury-strain vaccine in man indicate that the product will be safe for human immunization. Further experiments have to be made, however, to determine the optimal dosage and time of injection.

Unquestionably, the Flury rabies vaccine, with its high-degree long-lasting immunizing capacity and its relative lack of neuroparalytic factors, constitutes a major weapon in our armamentarium against infectious agents.

Blue-tongue

My laboratory has recently become interested in a hitherto exotic disease known as blue-tongue, since the disease is now well established in the sheep population of a number of our western States.^{37 38 39 40}

Blue-tongue is an infectious inoculable viral disease of cattle and sheep, characterized by catarrhal inflammation of the mucous membranes of the mouth, nose, and intestines, and often associated with inflammation of the coronary bands and sensitive laminae of the hoofs. The swollen and inflamed mouth and tongue take on the purple or dirty blue colour from which the disease derives its name. The disease is not transmitted by contact, but by biting insects. The infectious agent is an extremely resistant virus which remains viable for years in the fluid or dry state. At least four and possibly five or more immunologically distinct viral strains are currently recognized, yet they all produce the same clinical symptoms. Animals may carry the virus for weeks or even months after recovery, and are immune to subsequent infection with the homologous viral strain.

For many years blue-tongue was thought to be strictly an African disease. It has now occurred in Cyprus, Israel, and Turkey, and more recently in the western United States.⁴¹

Of the laboratory hosts commonly used for experimental work, only suckling white mice, injected intracerebrally, and developing chick embryos are apparently susceptible.

The blue-tongue vaccines in use to-day are based on the original findings of Alexander and his associates^{42 43 44 45} that any strain of the blue-tongue virus apparently will grow in the developing chick embryo provided careful attention is paid to the temperature of incubation.

By serial passage through chick embryos, virulent strains of blue-tongue virus are rapidly attenuated to a point where practically no clinical reaction is produced in sheep. As would be expected, each virus strain must be studied separately to determine optimal conditions, but attenuated strains retain their full antigenic capacity for inoculated sheep, provided the chick-embryo material used for vaccine is from a relatively low serial passage level. This is an extremely important point in the preparation not only of blue-tongue but of practically all other chick-embryo-propagated and attenuated vaccines. Long-continued passage in the foreign host—chick embryo in this case—will bring about over-attenuation of the agent to a point where it ceases to be a good immunogenic antigen. Thus great care must be taken at all times to use seed virus of the proper serial passage level in order to obtain an effective vaccine.

Alexander and his co-workers,^{44 45} also made the important observation that once a strain of blue-tongue virus had become attenuated by chick-embryo passage its virulence could not be restored or enhanced by 11 consecutive serial passages in sheep. I might add that a similar observation has been made recently by my associate Koprowski with our rabbit-attenuated strain of hog cholera virus. Koprowski⁴⁷ was unable to restore or enhance the virulence of the virus for swine by passing the attenuated strain for 10 consecutive serial passages in that species.

The present blue-tongue vaccine produced at Onderstepoort⁴⁶ is a quadrivalent living chick-embryo-attenuated product. Four different virus strains are grown separately in chick embryos, then pooled. The resulting vaccine contains approximately 250 chick-embryo infecting doses for each strain per 1-ml. dose.

In a personal communication,⁴⁸ Dr. Alexander, of South Africa, has advised that a strain of California origin has been found, in preliminary tests, to be relatively avirulent for sheep yet remarkably broad in its antigenic coverage. In these early tests the California strain developed good protection in vaccinated sheep against subsequent challenge with three antigenically different Onderstepoort strains. If subsequent tests confirm these results the problem of producing a blue-tongue vaccine of polyvalent antigenicity will be made much simpler.

As with yellow fever and Flury-strain rabies vaccines, no satisfactory chemical or physical method has been found for inactivating blue-tongue virus without destroying its immunogenic properties.

Poliomyelitis

I will now discuss some of the problems facing us in preventing poliomyelitis. At once I wish to make it clear that gamma globulin (immune serum globulin) is not the answer to the problem of securing long-term protection against poliomyelitis. The various shortcomings of immune-serum-globulin therapy and the attendant difficulties have been discussed in detail elsewhere,⁴⁹ so I will refrain from doing so here.

Without doubt, there is only one thing to do, and that is to put all possible effort into developing a practical and safe vaccine which will give long-term and solid protection against the disease. The problem is tripled by the fact that there are at least three main immunological types of the poliomyelitis virus. However, it must be remembered that the problem is really no more complex in this respect than

the one faced by investigators in the field of veterinary medicine in developing vaccines against blue-tongue of sheep and foot-and-mouth disease of cattle. We recognize four and possibly five immunological types of blue-tongue, and apparently six different antigenic types of foot-and-mouth disease (at least three of European origin and three African). The problem was met in the case of blue-tongue with the polyvalent living chick-embryo-adapted vaccine already described; and in the case of foot-and-mouth disease by a polyvalent killed vaccine utilizing virus strains cultivated in explanted bovine tongue epithelium.^{50 51 52 53 54}

Regardless of whether we wish to prepare a living attenuated or an acceptable "killed" vaccine, the problem is resolved to one of finding a plentiful and satisfactory source of tissue in which the virus will readily grow to high titre. Furthermore, the tissue used must not offer a potential source of any other pathogenic infectious or malignant agent for man. For killed vaccines, multiple injections of which will undoubtedly be necessary to produce a good immune response, extra care must be taken to avoid possible anaphylaxis and organ-tissue damage. Any adjuvant used must likewise be thoroughly tested and shown to be free of any potential carcinogenic or organ degenerative activity before it is recommended for large-scale use in man.

Present Progress

Now let us see what progress has been made in solving the problem of developing a practical and safe immunizing weapon against poliomyelitis.

Prime credit must be given to Enders, Weller, and Robbins,^{55 56} who first demonstrated that poliomyelitis viruses not only propagate in cultures of human embryonic and adult non-nervous tissues but also produce a cytopathogenic change therein. This latter finding makes it possible to recognize the presence of virus without the need of resorting to animal inoculation. The subsequent discovery that various monkey tissues, particularly the kidneys, provide a good culture medium for all three antigenic types of poliomyelitis still further opened up opportunities for study in the field. This approach for studying various aspects of the poliomyelitis problem, such as virus detection, propagation, assay, antibody quantitation, preparation of diagnostic antigens, and experimental vaccines, has been used by many workers, including Salk and his associates at the University of Pittsburgh,^{57 58 59} Melnick at Yale,^{60 61 62 63 64} Syverton at the University of Minnesota,^{65 66 67 68 69} Rhodes at the University of Toronto,^{70 71 72} and Evans at the University of Washington.^{73 74}

Recently, Salk⁷⁵ reported experiments in which he vaccinated 161 human volunteers with formalin-"killed" tissue-culture virus vaccines prepared from monkey kidney or monkey testes. An aqueous vaccine given intradermally induced an antibody response only against Lansing type 2 virus. However, a water-in-mineral-oil emulsion type "killed" vaccine inoculated deep intramuscularly was claimed to induce antibody response against all three types of poliomyelitis virus (Brunhilde, Lansing, and Leon). The vaccine contained only 0.1 ml. of tissue culture material of each of the three types. If it were true that such small quantities of inactivated virus would actually induce a primary antibody response in human beings, we would indeed have a very effective and practical vaccine available. Unfortunately, however, this is not the case. It should be noted that all of Salk's antibody titres were reported on sera taken a very short time after vaccination—never longer than four to seven weeks. Of greater importance is the fact that significant antibody titres were found in most of the vaccinated subjects prior to vaccination: 6 of 12 against type 2, 7 of 16 against type 3, and 15 of 15 against type 1. It is well known, of course, that it takes much less antigen to demonstrate a booster effect than it does to stimulate a primary antibody response in persons who have never had previous exposure to the agent. Thus analysed, Salk's results cannot be regarded as too favourable or impressive.

Need for a Satisfactory Assay Method

The basic information which should be in hand before any vaccine is tested on a large scale in human beings is, quite frankly, not yet available. Even the first step of developing a satisfactory and reproducible assay method in experimental animals has not been taken. None of the tedious and laborious—yet necessary—qualitative and quantitative tests in suitable experimental animals that are usually required of any new vaccine before it is used in either human or veterinary medicine have been performed. Such basic tests are absolutely essential to evaluate a vaccine.

Caution must be taken to prove that a vaccine contains a sufficient amount of antigen to make it a good immunizing agent rather than a sensitizing agent. In studies carried out with killed Rocky Mountain spotted fever and epidemic typhus fever vaccines, we found⁷⁶ that preparations which did not contain enough antigen to produce a good immunogenic vaccine actually sensitized the vaccinated animals and made them more susceptible to challenge than the non-vaccinated controls. Thus, if adequate quantitative studies are not carried out on proposed poliomyelitis vaccine preparations to ensure that they actually contain a sufficient amount of antigen, more harm than good could result from their use.

The recent findings of Scherer, Syverton, and Gey⁷⁷ that all three types of poliomyelitis viruses can be grown in a strain of human malignant cells (Héla strain) raises the question of whether such cultures could be used without danger as a source of vaccine. This question cannot be answered with certainty at present, but I am sure that no one would recommend the use of neoplastic cells or tissue as a medium for producing poliomyelitis vaccine.

At this point, I wish to state that there is no question whatsoever about the merit of tissue culture for research purposes. It can be gainfully used for many problems, such as isolation, identification, and propagation of infectious agents, quantitation of antibodies and assay of infectious agents by the neutralization technique, and for the development of virus variants that may be adapted to new and ordinarily non-susceptible hosts. My laboratory is particularly interested in the latter application because of its almost unlimited possibilities. A mouse-adapted variant strain of type 1 poliomyelitis virus (Mahoney strain) was derived from tissue culture material by Li and Schaeffer.⁷⁷ Further observations concerning the pathogenic spectrum of this strain are awaited with great interest because of the rapidity with which this virus apparently lost most of its virulence for intracerebrally inoculated monkeys.

Two Promising Leads

Returning to the problem at hand, a review of the literature indicates that the two most promising leads to follow for inducing immunity to poliomyelitis are the intramuscular and oral routes of administering the virus. I am of the opinion that probably the most logical and practical way to immunize against poliomyelitis is to use the natural portal of entry—the oral route—employing an attenuated living virus under qualitatively and quantitatively controlled conditions. This is the eventual goal of my laboratory, and we intend to continue our efforts in this direction until success is achieved. However, the road ahead is long and difficult, and we may have to content ourselves temporarily with a less satisfactory product until the present obstacles are overcome. My associates Koprowski *et al.*^{13 14} have carried the work a step further along the way by feeding a living rodent-adapted poliomyelitis virus to two sizable groups of human volunteers. The virus consisted of a suspension of brain and spinal-cord tissues of cotton-rats infected with the TN strain type 2 poliomyelitis virus, which had been shown to have lost much of its intracerebral virulence for monkeys. The individuals were observed carefully: none had a temperature rise or any sign of illness. Most of them became intestinal carriers of the virus for a

short time. Practically all of them who were not initially immune developed neutralizing antibodies to the homologous Lansing type virus, but not to the heterologous Brunhilde type.

While I believe the use of living attenuated virus offers the most logical and hopeful approach towards solving the poliomyelitis problem, I do not believe that the virus should be propagated in infected mammals or in infected mammalian explanted tissues until other approaches have been exhausted. Thus I am not in accord with Sabin's recent suggestion⁷⁸ of possibly using avirulent or "non-paralytogenic" poliomyelitis strains cultivated in monkey non-nervous tissues as a source of living attenuated vaccines for human use. Those responsible for producing vaccines on a large scale recognize that such infected mammalian tissues present the potential danger of being contaminated with other viruses or microbial agents which are infectious for man. These include lymphocytic choriomeningitis, infectious hepatitis, Sabin's B virus, encephalomyocarditis, tuberculosis, etc. The cost of testing to determine adequately the purity, safety, and identity of the product would be exorbitant if not prohibitive. These charges alone would tend to defeat our purpose of producing a practical vaccine that everyone can afford.

Without doubt the poliomyelitis problem would be greatly simplified if we could use the developing chick embryo as culture medium. The chick embryo is one of nature's best tissue-culture media, with the prime advantages of always being readily available in unlimited quantities, easily handled in the laboratory, relatively inexpensive, and, above all, free from micro-organisms potentially pathogenic for human beings. Furthermore, since the chick embryo is not a natural host for poliomyelitis virus, variant strains established in this medium will not regain their original virulence for men.

The MEF1 Strain of Virus

As you know, our faith in this approach already has been rewarded by the successful adaptation of the MEF1 strain Lansing type virus to the chick embryo.^{48, 79, 80, 81} In this instance the variant was obtained by carrying the virus for at least 119 consecutive serial passages in suckling hamster brain tissue.⁸² I am fully aware that the task of getting the Brunhilde and Leon strains similarly adapted is not an easy one, but I am confident it can and will be done. The successful fulfilment of this assignment will be worth whatever "blood, sweat, and tears" have to be expended. All I can say at present is that progress is being made; not as fast as we would like to have it, of course—yet we are advancing.

At least five separate substrains of the MEF1 virus have now been carried through more than 100 consecutive serial passages in chick embryos. LD₅₀ titres of the infected chick embryo suspensions, measured by intracerebral titration in mice, range from 10⁻⁵ to 10⁻⁶ or slightly higher. The major changes in the properties of the virus brought about by adaptation to the rodent host are retained essentially unchanged in the avian host for a considerable number of passages. However, there is evidence that long-continued passage in chick embryo results in gradual loss by the virus of some of its original antigenic characteristics for primates. Thus, as in yellow fever, Flury-strain rabies, and blue-tongue viruses, great care will have to be taken to use only those chick-embryo passage levels which secure optimal results as a vaccine.

One of the unusual properties of the adapted MEF1 strain of virus is that the chick embryo shows little, if any, sign of infection. Embryos do not die from acute infection, as so often happens with other neurotropic viruses, and the great majority die just prior to hatching time. Maximal growth of the virus apparently takes place in the embryo itself. The optimal time to harvest the infected tissues is on the fourth or fifth day following inoculation.

At present we are studying the antigenic characteristics of certain substrains of the chick-embryo-adapted virus in

relation to their intracerebral safety and immunogenic capacity by the intramuscular and oral routes for primates—particularly cynomolgus and rhesus monkeys and chimpanzees. These studies are still in their infancy. However, we have demonstrated that experimental vaccines, of little or no "paralytogenic" and no lethal effect when injected intracerebrally into rhesus or cynomolgus monkeys, produce an immunogenic response indicated by neutralizing antibodies when given intramuscularly but not orally to these same hosts.^{79, 83} The same vaccines, whether given intramuscularly or orally, apparently activate a good antigenic response in chimpanzees.⁷⁹ Neutralizing antibodies in significant titres appeared by the 14th day and persisted through the 127th day. Oral revaccination with the same preparation on the 127th day gave a good booster effect to three out of four chimpanzees. These studies are being continued and extended.

Conclusion

In summary, I wish to emphasize that from a practical standpoint the use of living attenuated viruses as immunizing agents is unquestionably the best method for securing long-lasting and safe protection. Many people are still uncertain about accepting the use of living virus vaccines in preventive medicine, yet the fact remains that the outstanding successes of specific immunization have been with living attenuated virus vaccines: smallpox, yellow fever, rinderpest, rabies, and blue-tongue. Present evidence indicates that attenuated living virus vaccines induce immunity through the same mechanism used by nature: by producing inapparent subclinical infections. Both the virus and the host are factors to be considered in bringing about immunizing infections, but by suitable qualitative and quantitative procedures we now are able to control changes in virus properties and make them suit our needs.

Finally, I wish to repeat that a tested practicable vaccine for poliomyelitis has not yet been produced. Furthermore, no one can now predict when such a practical, safe, and satisfactory vaccine will become available. It would be useless to make such a prediction now, because a tremendous amount of basic information still must be gained. However, I am optimistic. The tools and methods are at hand to do the job. I am confident that before long we will succeed in propagating all three major types of poliomyelitis virus in the developing chick embryo, and achieve our goal of a living attenuated virus vaccine which will confer durable protection against poliomyelitis. Thus another potent weapon will be added to our arsenal against disease.

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The *Annals of Eugenics*, which was founded by Karl Pearson in 1925, has now changed its title to *Annals of Human Genetics*. The numbering of volumes remains unaltered. The journal is edited by Professor L. S. Penrose, F.R.S., and published by the Cambridge University Press.

THE DIAGNOSIS AND TREATMENT OF HYPOPITUITARISM

BY

S. R. F. WHITTAKER, M.D., F.R.C.P.
Physician, Warwick Hospital

AND

T. P. WHITEHEAD, F.R.I.C.
Biochemist, Warwick Hospital

During the past three years we have studied nine cases of hypopituitarism and endeavoured to find a satisfactory scheme of treatment using A.C.T.H. and cortisone in addition to preparations of the thyroid gland and testosterone. In the course of this investigation we have been struck by the distressingly long period, sometimes extending over a number of years, that may elapse between the onset of symptoms and the establishment of the diagnosis. Despite the classic papers of Sheehan and his associates (Sheehan, 1939; Sheehan and Summers, 1949; Cooke and Sheehan, 1950), it appears that the syndrome of hypopituitarism may still pass unrecognized for a considerable time after the patient has sought medical aid, possibly owing to the confusing variation in the impairment of function of the thyroid gland, the adrenal cortex, and the gonads that may exist in different patients.

Since the treatment of hypopituitarism with A.C.T.H. and cortisone promises to be a considerable advance on previous methods, an early diagnosis assumes more importance, and we feel it worth while to record some observations on the clinical picture, a brief account of the investigations likely to be of value in establishing the diagnosis, and the results of treatment with A.C.T.H. and cortisone, sometimes combined with other hormones, for periods up to two years.

Material.—This study is based on seven women and two men whose ages ranged from 32 to 63 years, with an average of 47. Five of the women (Cases 1-5) had suffered from severe haemorrhage after childbirth, and a post-partum necrosis of the pituitary gland was likely. The exact nature of the pathological process damaging the pituitary gland is unknown in the other four cases, although one patient had been treated for syphilis some years previously (Case 9). One patient died during the course of the investigation (Case 1).

Clinical Picture

The difficulties of an early diagnosis are perhaps illustrated by the fact that nine patients between them paid over 30 visits to different hospitals or hospital departments before the diagnosis of hypopituitarism was finally made. The average duration of symptoms was over 10 years, and, unless there was a clear history of haemorrhage during childbirth followed by absence of lactation and scanty periods or amenorrhoea, it was difficult to determine accurately the date of onset of the disease. The clinical picture varied from patient to patient, but the following features were the most constant.

Tiredness.—All patients complained of feeling tired, and it was usually this symptom that first prompted them to visit a doctor. A diagnosis of anaemia was often made, and some tonic prescribed. The tiredness continued but rarely became incapacitating, and after a time these patients became accustomed to feeling tired and struggled on as best they could, aware that there was something the matter