March 22, 1957.

SPECIFICATION

The currently used poliomyelitis vaccinations by killed virus (Salk vaccine) do not confer on the patient the disease resistance which is conferred by the disease itself. This type of vaccination may greatly raise in the vaccinated individual the titer of the circulating antibody against the polio virus. But if such an individual, who had no previous contact with live polio virus, is infected through the oral route by live polio virus so that the intestinal mucosae are invaded by the virus and the intestinal phase of the disease is initiated, this phase of the disease has about the same intensity and duration as in an unvaccinated individual. Therefore, a population which has been immunized through Salk vaccination continues to be subject to the intestinal polio epidemics. Those individuals in the population who have been Salk vaccinated and have a high titer of circulating antibody will be protected by this antibody against the spreading of the disease from the intestinal tract to the nervous system. Individuals, however, who are not Salk vaccinated - even though they live within a population where most of their fellow citizens have been vaccinated - have about the same chance of paralytic polio as individuals who live within a comparable unvaccinated population.

In contrast to a person who had never had contact with live polio virus but has been Salk vaccinated, a person who has been infected through the oral route with live polio and who has gone through the intestinal phase of this disease, has thereby acquired disease resistance not possessed by the former. If the latter is infected with polio through the oral route, the intestinal phase

Witnessed - March 22, 1957:

Carol Andrén

Insert on Page 3 -

In order to perform the test ovalbumine (or diphteria toxoid) is substituted for the killed virus in the composite vaccine, and antiserum against ovalbumine (or diphteria toxoid) is substituted for antibody against the killed virus in the composite vaccine. The test consists in determining whether this substituted composite vaccine, if injected in a given manner, evokes hyper-sensitivity of the delayed type.

Whether this substituted composite vaccine evokes hyper-sensitivity of the delayed type can be tested in either of two ways:

Witnessed - March 23, 1957.

Carol Andrén

of the disease will be very much shorter than in the former one who, if infected with polio through the oral route, will go through the intestinal phase of the disease with approximately the same intensity and duration as an unvaccinated person who had not been previously exposed to polio.

The polio virus is one of the viruses of a group called D.R. viruses, the members of which have the following characteristics in common:

a) there is an initial phase of the disease in which the intestinal mucosa or the mucosa of the respiratory tract (including the nasal passages) are affected;

b) the disease causes lasting disease resistance in the sense that on a subsequent infection the initial phase is either completely suppressed or weakened and shortened in its duration.

Vaccination with the killed virus in the manner which is currently practiced in the case of poliomyelitis (Salk vaccine) - even though it may lead to a high titer of circulating antibodies - does not cause lasting disease resistance with respect to shortening the initial phase of the disease in which the intestinal mucosa or the mucosa of the respiratory tract are affected.

According to this invention it is possible to produce such disease resistance in the above defined group of viruses over and above the kind of immunity conferred on the patient by the circulating antibodies by vaccinating the patient with a composite vaccine. This composite vaccine contains killed virus and either an antibody which is specific for the killed virus, or an adjuvant, or both, and it produces disease resistance which is specific to the killed virus used if injected in the manner described below.

Witnessed -March 22, 1957

Carol Andren

The composition of the vaccine and the manner in which it is injected will produce disease resistance in every case when the following conditions are satisfied: if the killed virus is replaced by about the same quantity of either ovalbumine or diphteria toxoid, and if the antibody against the virus is replaced by an antibody against ovalbumine or diphteria toxoid, and if the composite vaccine which is thus substituted is injected in a manner in which a high degree of hyper-sensitivity of delayed type is evoked against ovalbumine or against diphteria toxoid, respectively, then according to this invention the composite vaccine injected in the same manner, will produce disease resistance.

This provides a comparatively simple test that permits us to determine in advance whether the given composite vaccine containing killed virus, if injected in a given manner, may or may not be expected to produce disease resistance. Whether either test which is carried out by substituting either albumine or diphteria toxoid in the above stated manner for the killed virus produces hyper-sensitivity of the delayed type can be tested in either of two ways:

Insert

a) if the composite vaccine containing ovalbumine or diphteria toxoid does not evoke circulating antibodies, then the presence of delayed hyper-sensitivity can be determined by injecting ovalbumine (or diphteria toxoid) into the skin of the test subject;

b) if the composite vaccine containing ovalbumine (or diphteria toxoid) evokes an early appearance of circulating antibodies in the test individual, then the absence of hyper-sensitivity may be determined by transferring lymphocytes to the second test individual and then skin test the second test individual with ovalbumine (or diphteria toxoid).

Witnessed March 22,1957
Carol Andren

Leon B. Lund

It is established that delayed type of hyper-sensitivity can be evoked by injecting ovalbumine (or diphteria toxoid) mixed with antibody (about a four-fold excess of antibody may be used) and evoke delayed type hyper-sensitivity against ovalbumine (or diphteria toxoid) without giving rise to an early appearance of circulating antibodies. In these cases the appearance of delayed type hyper-sensitivity subsequent to the injection, can be ascertained by skin testing the injected test subject. Experiments of this type have established that intracutaneous injection of 30 microgram of diphteria toxoid or ovalbumine in the form of an antigene-antibody precipitate containing a four-fold excess of antibody, will produce delayed type hyper-sensitivity when injected intracutaneously (in 10 portions of .1 cc each).

Such antibody precipitates mixed with complete Freund adjuvant injected in adequate quantity subcutaneously, will also evoke delayed type hyper-sensitivity. In neither of these two cases is there an early appearance of circulating antibody against ovalbumine (or diphteria toxoid).

If an excess of antigene ~~of~~ over an antibody is used and if an adequate amount is injected subcutaneously mixed with complete Freund adjuvant, circulating antibodies against ovalbumine (or diphteria toxoid) will be produced. This makes it difficult to determine by direct skin test on the injected individual that hypersensitivity of the delayed type against ovalbumine (or diphteria toxoid) is produced. This, however, can be shown to be the case by transferring lymphocytes to a second test subject and by skin testing the second test subject.

According to this invention, ~~injecting a composite vaccine containing a killed virus of the D.R. group and in~~ in case of the viruses of the D.R. groups and in particular the polio virus and/or

Witnessed - March 22,1957

Carl Andren

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Leon B. Zand

particular injecting a composite vaccine constituted as described above and injected in the manner described above but substituting the killed virus for ovalbumine and substituting antibody against the killed virus for antibody against ovalbumine and using the above mentioned proper route of injection in each given case, will lead to disease resistance that will approximate the disease resistance that ensues following an intestinal infection by the live virus.

Thus injecting intracutaneously 30 microgram of killed polio virus (Salk vaccine) mixed with antibody against the killed polio virus (four-fold excess), will lead to a high level of disease resistance against polio approximating the disease resistance conferred by an intestinal infection with live virus.

If an individual who has been Salk vaccinated and as a result of this vaccination has high titer of circulating antibodies against the polio virus, is also made disease resistant by being inoculated with this composite vaccine, then he is - in case of an oral infection with live polio virus -

a) protected against the spread of the virus from the point of entry to the nervous system; and

b) the intestinal phase of the disease will be about as weak and as short as if he had had a previous intestinal infection with live virus.

In preparing the composite vaccine which contains antibody but does not necessarily contain adjuvant, ~~and which must be injected intracutaneously,~~ it is desireable to use antibodues contained in human sera rather than antibodies obtained from animal sera.

Witnessed -March 22,1957:

Carol Andrén

Carol Andrén

The amount of antibody against the virus present in the human serum can be essayed as follows: One prepares a series of samples using the ~~same~~ same quantity of virus in each sample with increased amounts of ~~serum~~ antiserum in subsequent samples. Each subsequent sample which is injected, may contain twice as much antiserum as the preceding sample. Each of these samples is injected ~~intra-~~ ~~dermally~~ into a monkey that has not been previously exposed to polio. The monkeys which have been injected with a sample that contained low amounts of serum will respond with early production of circulating antibody. The sample injected in the first of the monkeys of the series which does not respond with early production of circulating antibody, contains a slight excess of antibody over the killed virus and thus determines the antibody titer of the serum ~~used~~ for the purposes of preparing the composite vaccine. The remaining monkeys in the series that have been injected with samples containing higher amounts of antibody serve as controls. They must show no early formation production of circulating antibody if the essay is to be valid.

Such an essay permits us to prepare a composite vaccine composed of killed polio virus and a slight excess of antibody against the polio virus. Such a composite vaccine if injected in adequate amounts intracutaneously will confer disease resistance on the patient.

By injecting subcutaneously an adequate amount of killed polio virus (Salk vaccine) mixed with complete Freund adjuvant, both circulating antibody and disease resistance can be induced at the same time. The same holds if antibody is present in this composite vaccine containing complete Freund adjuvant, it is preferable to have an

Witnessed -March 22, 1957:
Carol Andren
Carol Andren

provided there is an excess of Salk vaccine over the antibody against killed polio virus. A five-fold excess of killed polio virus over the antibody is adequate to produce high titer of circulating antibodies, if the amount injected is sufficient to produce a high level of disease resistance.

Concerning the state of the art in respect to evoking hyper-sensitivity of the delayed type I refer to Uhr, J.W., Salvin, S.B. and Pappenheimer, A.M.Jr., Journal of Experimental Medicine, Vol. 105 (1957), and to Lawrence, H.S., and Pappenheimer, A.M.Jr., Journal of Experimental Medicine, Vol. 104, (1956).

Witnessed - March 22,1957:

Carol Andrén

Carol Andrén

C L A I M S:

I claim -

1) A therapeutic preparation comprising a killed virus of the D.R. group of viruses and antibody against the said virus - the said therapeutic preparation representing a composite vaccine suitable for intracutaneous injection;

2) A therapeutic preparation comprising a killed polio virus and antibody against the said polio virus - the said therapeutic preparation representing a composite vaccine suitable for intracutaneous injection;

3) A therapeutic preparation comprising a killed virus of the D.R. group of viruses and complete Freund adjuvant (comprising an oil-water suspension and mycobacteria) - the said therapeutic preparation representing a composite vaccine suitable for injection;

4) A therapeutic preparation comprising a killed polio virus and complete Freund adjuvant (comprising an oil-water suspension and mycobacteria) - the said therapeutic preparation representing a composite vaccine suitable for injection;

5) A therapeutic preparation comprising a killed virus of the D.R. group of viruses, antibody to the said virus and complete Freund adjuvant (comprising an oil-water suspension and mycobacteria) - the said therapeutic preparation representing a composite vaccine suitable for injection;

6) A therapeutic preparation comprising a killed polio virus, antibody to the polio virus and complete Freund adjuvant (comprising an oil-water suspension and mycobacteria) - the said therapeutic preparation representing a composite vaccine suitable for injection.

Witnessed - March 22, 1957.

Carol Andrén

Leo Ludwig

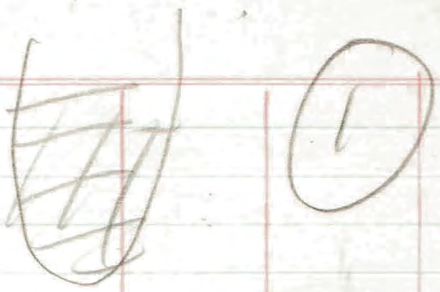
As a clarification of the foregoing text relating to Agents for Producing Disease Resistance, I may add the following: The DR agent described will produce the desired effect best if it is injected intradermally, or, if injected subcutaneously it should be injected together with an adjuvant such as for instance Arlacel plus Bayol F. The expression "relevant antigen" on page 4 must be taken to mean surface antigens of the infectious agent, and the expression "relevant antibodies" must be taken to mean antibodies against the surface antigens of the infectious agent.

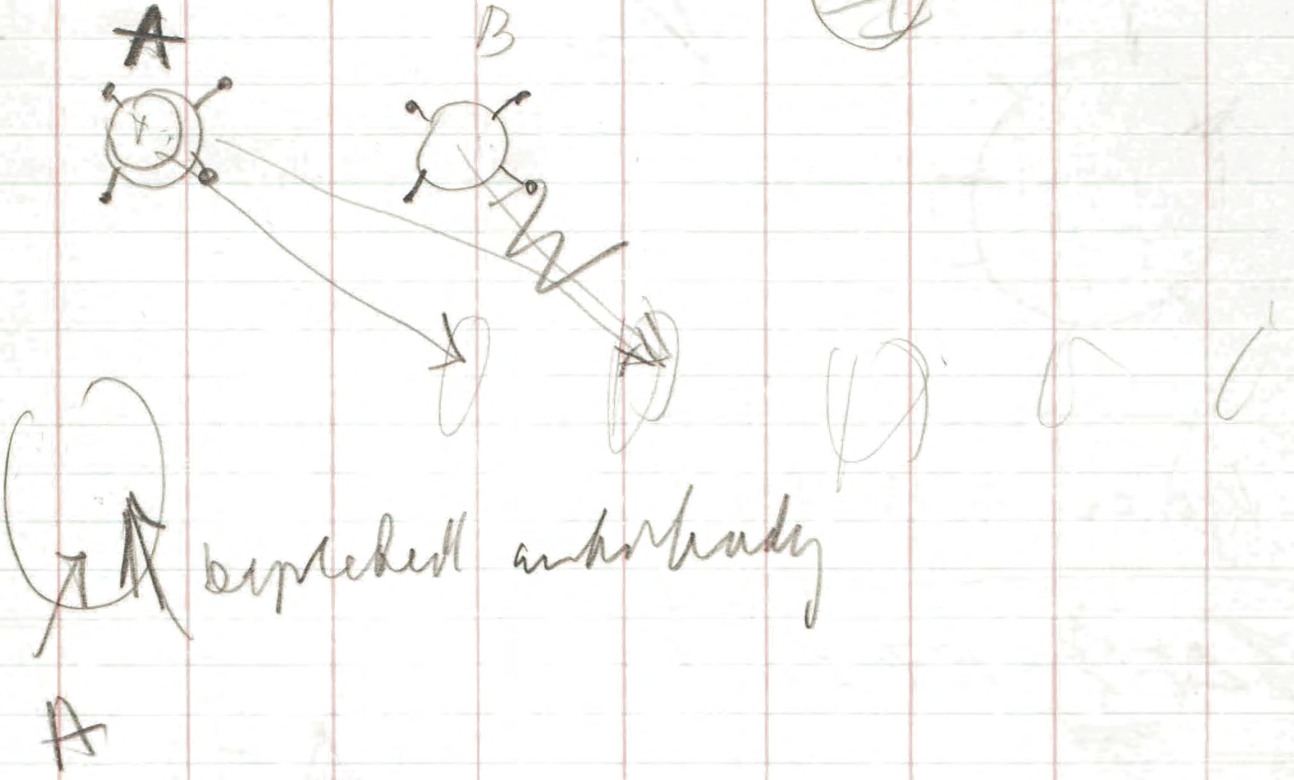
Witnessed - January 18, 1957.

Carol Andrén

(a) Lerman Febr 25/57

a



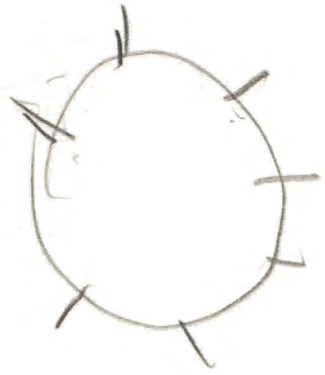


$I a_1$ Does fraction I of antibody a sterically hinder absorption of another fraction II of a

(A)

high constant more
less stable

$\frac{K_1}{K_2} = K$



$K_1 C_1 C_2 = K_2 [C_1 C_2]$

$\Delta F^0 = RT \ln K$

ΔH

Cyclic Pump Kaust

$CH_3 (CH_2)_n CO_2^-$

Δ

$r = A e^{-\frac{E_a}{RT}}$

$r = e^{+\frac{\Delta S}{R}} \cdot e^{-E_a/RT}$

$r = e^{\frac{\Delta F}{RT}}$

Please return to Sa

$$\frac{dy}{dt} = Q\frac{P}{cy+P} - \frac{y}{\tau}$$

$$\left.\begin{aligned} \frac{t}{\tau} &= x \\ Q\tau &= A \\ \frac{c}{P} &= B \end{aligned}\right\}$$

$y = 0$

$t = 0$

$$\boxed{\frac{dy}{dx} = \frac{A}{By+1} - y}$$

for $x = 0 \quad y = 0$

$$\frac{dy}{dx} = \frac{A - y(By+1)}{By+1}$$

$$\int_0^y \frac{By+1}{A - y(By+1)}\,dy = x$$

wanted, if possible
y expressed as
function of x

$$P = \frac{Q}{(n-1)}\,\frac{c}{n}$$

n, positive > 1

Mrs Mann Ext 3789 Room 381

Blocked between Acetyl ~~orn.~~ ornithine and ornithine

‖ O ⊤ C → A (E) W Strain 1163/37

In Chemostat org. transfer:

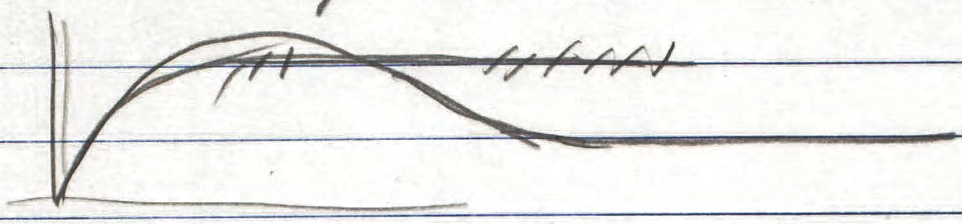
τ = 12 h doubling

τ = 1.2 h doubling

sometimes more enzyme than would grown without arginine

Wild type grown first time in ~~agar~~ arginine wash!

no arginine medium



160/37

| → O → C | → A

~~Wild type or W 160/37~~

When we transfer from arginine medium to citrulline medium enzyme initially rises sharply.— same when we transfer

doubling time

2 in 10 min 50x144 = 72 min