I am continuing now to read from the report from the memorandum of June 29, 1955 to the NFIP Committee on Virus Research. I am on page 5, and point number 2. The studies on chimpanzees led me to believe that parental inoculations of attenuated viruses—let me say here that this refers to inoculation intramuscularly, subcutaneously, could produce a so-called closed immunogenic infection because no virus was excreted in their stools after intramuscular injections of virus which produced antibodies. In human beings, however, it was found that the small amount of virus which finds its way into the circulation from the site of intramuscular injection of about ten to the sixth. That is one millionth tissue culture infective doses can quickly localize in the lower alimentary tract where it can multiply sufficiently to be excreted in amounts of ten thousand to one hundred thousand tissue culture doses per gram of feces over a period of 28 days.

I want to digress here. I want to call attention to the fact that all of these studies were quantitative. That only quantitative studies had significance. Not the easy way out. Was it present, was it absent. That means nothing. And that is why our work was so meticulous, and required such a tremendous—a lot of staff because everything was quantitative.

I am going back to this. This experiment has been carried out thus far only with the type 3 virus. The amounts of virus, ten to the 4.5 to ten to the 6.5. That is 3200 to...
the 3 million 200,000 which readily infected by mouth failed to multiply or to produce antibody after intramuscular injection except in one individual with localization of the virus in the alimentary tract. Therefore, unless and until a variant is found which has the reverse properties, namely the capacity to multiply preferentially in the skin or muscle of human beings it would appear best to concentrate future studies on the alimentary rather than the parentral routes.

As an aside here, now this is a tremendously important observation because at that period we had to consider the possibility, why take a chance on an attenuated virus that would spread from person to person when it was given by mouth and multiplied in the intestinal tract. If you could have one that you could give by subcutaneous or intramuscular injection that would immunize and presumably that would produce resistance in the intestinal tract, a lot of if's. Well, it might be preferable. But then it turned out that it was no good in human beings. When you came to test it in human beings because the quantities which produced infection when given by mouth wouldn't do anything when they were given intramuscularly except in one person in whom it localized in the alimentary tract and then multiplied. So this served to exclude the parentral approach and to concentrate on the oral. you see. It wasn't some pre conceived notion or a prejudice or an attempt to imitate the natural course of events. It just was no other way. It didn't work.
Q Now that is important.
A It didn't work. Now I am returning to reading from the summary. Point number 3. The smaller doses of virus. I am going to read logarithms here.
Q Okay.
A Ten to the 4.5 TCD$^{50}$ and in two individuals also ten to the 6.5 TCD$^{50}$ failed to multiply or induce antibody formation in volunteers possessing irregularly or barely detectable or small amounts of naturally occurring or experimentally produced antibody. The feeding of larger amounts of virus ten to the 6.5 to ten to the 7.5 did induce an antibody rise in volunteers with naturally acquired or experimentally produced low levels of antibody but the amount of virus excreted was small. And the duration of virus excretion was less than in volunteers without antibody. It is obvious therefore that quantitative studies in human beings designed to answer questions regarding the amount, duration and character of virus excretion cannot be carried out in volunteers with antibody. Whether or not antibody produced by a killed virus vaccine would have the same effect as a natural or experimental infection cannot be answered without a test. A test which incidentally needs to be performed but with simultaneous controls in individuals without antibody.

Number 4. In four of fourteen infected individuals the type 3 virus produced only a transitory antibody response and in most of the others the antibody persisted at a low level. Dr. Hammond has observed a similar phenomenon in naturally occurring symptomless type 3 infections in the
Philippines. I want an aside here. This is terribly important because of certain fixed ideas that virologists have that there must be a correlation in natural infection between demonstrable antibodies and resistance. And it is obvious that that is not the case. Four of fourteen produced no antibodies, or transitory antibody response. And this was also then, as I say, observed by Hammond in naturally occurring type 3 outbreak in the Philippines.

The volunteers with a transitory--that means it was present for a while and then disappeared--antibody response either failed to become infected or had a low grade infection with another transitory antibody response when they were given a second dose of the same virus three months after the first. In view of this it becomes important to determine whether these results are peculiar to some attenuated strains of type 3 virus but not to others. The effect of swabbing this type of virus directly on the throat also remains to be determined.

Number 5. During the course of these studies, it was observed that the volunteers who swallowed the larger amounts of virus had an infection of the pharyngeal wall as well as of the lower alimentary tract. And that those with a good pharyngeal infection had a better antibody response. Seven volunteers--three for type 1, and four for type 2--were available for a test of the effect of swabbing virus directly in the throat. This was done by dipping a cotton swab in undiluted culture fluid and after squeezing out the excess fluid in the tube the swab was lightly rubbed on several
areas of the posterior pharyngeal wall. A prompt and good symptomless pharyngeal infection with good antibody production was set up in all. Viremia, tested from the third to the fourteenth days was absent in all. And no significant amounts of virus appeared in the mouth.

I want to mention an aside here that subsequently this became a subject of some controversy when different people began to do tests for viremia with the attenuated strains that I supplied. The upshot of it was that everything is quantitative. That if you used very much larger amounts of blood you could demonstrate a trace, you see. But of course there is a great difference between having to use very large quantities of blood and not finding any in ordinary amount.

What was particularly—I am back now to the summary—what was particularly noteworthy was that in the type 2 group virus excretion in the stools was minimal in amount and of relatively shorter duration. In the type 1 group more virus was excreted than in the type 2 group but generally less was excreted by those who swallowed the virus.

In view of this observation future plans will include a test on the effect of swabbing the virus on the throat as well as the effects of swallowing doses containing about one million and ten thousand tissue culture doses of virus. Thus, nine volunteers would be used for the initial testing of any one strain. Three for ten to the sixth. That is one million and three for ten thousand. Ten to the four by mouth and three for swabbing with undiluted culture fluid.
I want to give an aside again. Even though swabbing on the throat could not be conceived as a practical way of administering mass administration of vaccine, we nevertheless had to learn one had to study what was happening as a basis for ultimate policy decisions.

Now when the three optimum strains had been selected, it would also become important to determine the effect of swabbing each of the three types on a separate portion of the posterior pharyngeal wall like plating three different bacteria culture on separate portions of a titer plate.

Next point. Change in viral population after multiplication in alimentary tract of chimpanzees and human beings and question of harmful mutants. No bacterial or viral population is so homogeneous that by the use of appropriate selective media it is not possible to demonstrate and segregate a varying number of individuals which are different in some property from the major portion of the population. Previous studies from this laboratory have demonstrated that variants that are dominantly avirulent for monkeys by the intracerebral route contain one particle—it should perhaps be one infectious dose in about one million to ten million infective units that can propagate with varying degrees of efficiency in the brain stem neurons of the monkey and thus produce paralysis after intracerebral as well as after intraspinal injection. Repeated passages of such a strain using large inocula in monkey kidney cultures does not alter this proportion. Although the particle with these special properties can be selectively segregated by
intracerebral injection in large numbers of monkeys of amounts of tissue culture fluid containing more than a million TCD$^{50}$ of virus. A number of such isolates have been shown to lack paralytogenic activity after extraneural injection in monkeys and also after direct spinal inoculation in chimpanzees. The virus excreted in the stools of chimpanzees and human beings which received—should be who received—the type 3 Leon virus is in this respect no different from that propagated in monkey kidney tissue culture. Among the type 1 viruses the Enders variant of the Brunhilde strain and two derivatives of the Mahoney virus obtained by Lee and Schaeffer have after feeding to chimpanzees in this laboratory yielded progeny in the stools which, from the point of view of intracerebral virulence for monkeys, were not different from the viruses propagated in tissue culture. Note. The Enders virus is the most virulent of all in spinally inoculated monkeys.

This is something that we didn't mention before. It had low virulence on intracerebral inoculation. But when put into the spinal cord it was the most virulent of all. The other strains didn't—

Q  Was it more virulent than the Mahoney?
A  Oh yes. Derivatives. Not the original now. Virulent one. But you see of the various attenuated strains, that was the most virulent in spinally inoculated monkeys. And the Lee and Schaeffer variant of the Mahoney virus which are less virulent monkeys are poorly immunogenic in chimpanzees.

Our own Mahoney attenuated strain has regularly yielded progeny in stools of both chimpanzees and human volunteers with
a higher proportion of intracerebrally virulent particles than is contained in monkey kidney tissue culture fluid. However, these particles derived from the stools have also been shown not to be paralytogenic for monkeys by extraneural routes and for chimpanzees by the spinal route.

What I am talking about here—this is an aside—is that while you can demonstrate a change in one parameter, that when you apply the crucial test, there is no change. In other words it is not a question of is there change or no change. It is not a question of is it stable or not stable. The question, as I pointed out before, is how much and how far has it gone and what advantage if any does it have in multiplication in competition with the millions of other virus particles.

Intracerebral tests in monkeys on viruses derived from human throat swabs yielded the same results and indicated that the larger proportion of intracerebrally virulent particles in the stools was not due to greater vulnerability of the intracerebrally avirulent particles in the lower alimentary tract. That the intracerebrally avirulent particles in the—~that the intracerebrally virulent particles do not become dominant is evident from studies on different stool samples from chimpanzees and human beings who continue to excrete virus for long periods of time.

I want to stress an aside here. In other words, if they had a selective advantage, after long multiplication in the alimentary tract they should become the dominant virus, but they didn't.
Furthermore, a second passage in chimpanzees by means of stools did not produce virus with a higher proportion of intracerebrally virulent particles. It would appear therefore, that while in monkey kidney tissue culture intracerebrally virulent particles occur with a frequency of one in about a million to ten million, the incidence in the alimentary tract occasionally is as high as one in a thousand to one in ten thousand. The type 2 YSK virus behaved similarly in all chimpanzees. But this effect was observed in only two of six human volunteers. The virus derived from one of the two volunteers was not paralytogenic on subcutaneous injection in eight cynamologous monkeys nor after spinal injection of one million tissue culture doses in three chimpanzees.

Two naturally occurring type 1 viruses and one type 2 virus, when fed to chimpanzees yielded results similar to those obtained with our Mahoney and YSK strains. It is obvious that the best test for harmful mutants would be by spinal injection in chimpanzees. But this unfortunately cannot be done on a large scale. When it was done, however, no harmful mutants were found. It is possible that the tests for extraneural paralytogenic activity in monkeys may correlate better with the lack of virulence for the chimpanzee and thus represent a better practical measure of the appearance of harmful mutants in the alimentary tract than the intracerebral test in monkeys. Nevertheless, since there are strains which yield similar populations of virus particles in the alimentary tract, in monkey kidney cultures as regards intracerebral virulence
for the monkey. Such strains will be preferred if they prove to be otherwise satisfactory.

I think I will go a little longer and finish reading this.

Q Yes.

A The next paragraph is entitled Currently available attenuated strains requiring testing in human volunteers. Do you have enough there?

Q Oh, we have enough.

A Alright. There are two type 1 strains. Now excuse me. I am saying an aside here because this is after weeding out. This was a summary of the status in the middle of 1955. So I say there are two type 1 strains at that time. The Lee Schaeffer derivative of the Mahoney strain which has the least neuronaltropic activity in monkeys of all strains tested thus far but which in a dose of ten million TCD$^{50}$ produced a delay a minimal antibody response in chimpanzees. And (b) the P-15-53 New Orleans strain derived from a healthy child. It exhibits less neuronatropic activity in monkeys than our Mahoney strain which already has been tested in man and yielded a better antibody response in chimpanzees than the Lee/Schaeffer virus. One type 2 strain, Cincinnati FAF-117 and one type 3, Cincinnati Glen strain derived from healthy children are available for study. After a number of rapid passages in monkey kidney tissue culture and purifications by the terminal dilution technique both are still intracerebrally avirulent for monkeys but somewhat more active intraspinally than the
YSK and Leon viruses. Both produced a rapid and good antibody response after feeding to chimpanzees. These four strains can be tested in a total of 36 adult human volunteers. The type 3 Leon virus should be retested in a group of six volunteers to determine its effectiveness by swabbing on the throat.

The next item is titled Program of human studies after optimum strains have been selected. The following questions will require investigation after the best strain of each type has been selected.

(a) interference. My own studies on chimpanzees with all three types fed simultaneously and Koprowski's studies on human beings with different mixtures of type 1 and 2 indicate that interference may be expected. However, it is desirable to determine whether swabbing each type of virus on a separate portion of the pharyngeal wall may produce a different effect. It may also be well to follow the example of nature and feed only one virus at a time. For example, start with type 2; follow with type 3 in about three weeks; and finally feed type 1 in three more weeks, completing the series in six weeks.

These studies would have to be carried out in children without antibody for any of the three types of polio virus.

I want to interject an aside. Of course this was done. And what was found later interestingly enough is that three weeks is not enough to produce the best intestinal resistance to subsequent infection. And it was found by field studies that it is much better to provide at least two months for each type to multiply by itself and thereby to give the greatest
resistance to subsequent infection. However these were the original approaches. And was also chosen type 2 first and type 3 second and type 1 last based on what was previously found that some protection cross protection may exist and that even in nature the type 2 and type 3's no matter what they are in monkeys, give rise to much less paralysis than type 1. So, if you have to do them individually, let the type 2 and type 3 multiply and finally end up with the most important one where there will be no interference. And actually we found—again this is an aside. That three weeks was not enough. That type 3 multiplies—can multiply for six weeks, eight weeks. And if you feed type 1 it may not take off well. Type 3 may still interfere. But who can tell ahead of time. This we learned by studies. Now that was under "a" interference.

Under (b) I have best mode of administration. The oral route has an obvious practical advantage over pharyngeal swabbing. Unless the latter should prove to be superior with respect to regularity of persistant antibody response and minimal amounts of virus excretion.

(c) Effect in infants with placentally transmitted antibody. Here the effect of various levels of antibody must be studied on the infective capacity of the selected dose of virus.

(d) Effect in individuals with different levels of antibody resulting from inoculation with killed virus vaccine.

As an aside. When this was being planned, there were already millions getting killed virus vaccine. The purpose here will be to determine what levels of antibody if any could
completely prevent infection with a selected dose of virus. This study would have to be done on children who were known to have had no antibody for any of the three types prior to inoculation of the killed virus vaccine.

As an aside. This was ultimately achieved in an institution because natural infection, if they had natural infection before they got Salk vaccine, it didn't mean anything. It would be the resistance caused by the natural infection. Finally (e) Administration of the vaccine to progressively larger groups of children. This step would be indicated after the best method of administering the three optimum strains of virus had been determined. The purpose would be the ultimate determination of safety by actual field trials in groups ranging from a hundred to five hundred to one thousand etc., etc., etc. And there is an aside. Of course it finally got up to millions before decisions were made.

The final paragraph. This memorandum has outlined a program to be carried out in a series of consecutive steps. Each subsequent one to be determined by the results obtained in the preceding step. Some of the work here outlined can be completed in 1955 and 1956, but much of it will require study over a period of many years. The results of these studies as well as other events including the infectiveness and duration of immunity provided by killed virus vaccine and the potential availability to all the people in different parts of the world requiring protection will determine the ultimate progress of this work. As I have indicated elsewhere it would
be fine if it turned out that the information required for studies of immunization with attenuated viruses should not be needed. But, it would be a pity not to have this information in reserve for practical application when and where needed. Period.

Now let me say as a sequel to this there is a letter several months later of October 3, 1955 to Henry Coohm in which I report, a brief progress report on the search for strains possessing minimal pathogenicity for monkeys by the spinal route which can be included in the record. Additional observations on human volunteers. This is all 1955. Present status of the work and plans on chimpanzees rhinitis—Well this is something else entirely.

And this I think we may start with—

Q Yes we will start with that—yes, next time.

A And we will stop right here.

END OF TAPE