Interview with Dr. Albert Sabin
May 22, 1976
Side 1; Page 1

A Supplement to our discussion of May 14, May 15 in which the work covered by the semi annual reports of January to December of 1953 had been covered. The important observation mentioned there was that the MEFl mouse adapted virus from Dr. Olitsky's laboratory had been found by me to be non-scitopathic for monkey kidney cells in tissue culture. But, that without destroying the cells, without being scitopathic, it nevertheless imparted a resistance to super infection. I recalled at that time my publication in Science of work that I did very shortly after the publication of the discovery of Enders, Weller, Robbins of the capacity of polio virus to multiply in tissue culture in non-nervous tissue. And I wanted to know why did my own studies in 1936 not pick up this question. This phenomenon. So this is the publication, less than a page in Science of August 27, 1954 under the title "Non-scitopathogenic variants of poliomyelitis viruses and resistance to superinfection in tissue culture." Now I am going to--just a moment stop.

I am reading here from the publication in Science of August 27, 1954 on non-scitopathic variants of poliomyelitis viruses and resistance to superinfection in tissue culture.

Ever since unequivocal multiplication in non-nervous tissue was demonstrated by Enders, Weller and Robbins thirteen years after my own experiment in 1936 failed to show any multiplication in non-nervous tissues, it had been an intriguing
question why those 1936 experiments with non-nervous tissue—that is, human non-nervous tissue—had failed. My own recent experiments had demonstrated—incidentally, as an aside here I am modifying a little bit the reading to bring it more—

Q Sure.

A My own recent experiments have demonstrated that intracerebral passage in monkeys favors the segregation of variants that are devoid of scitopathogenic activity for fibroblasts growing out of monkey testicles in tissue culture but possess full scitopathogenic activity for epithelial cells derived from monkey kidney.

So that was a little difficult to understand. It still did it in monkey kidney although it didn't do it for fibroblasts and testicles.

Now more recently I have found that from the nervous system of infected animals it is possible to segregate variants that have no scitopathogenic effect on epithelial cells derived from monkey kidneys.

This again is an aside, pointing once again to the multiplicity of potentials in individual virus particles in a population of polio virus in a culture. Then I continue.

Since the MV strain of virus that was used in 1936—in the 1936 cultivation experiment, that had a large number of intracerebral passages—I have just noticed a mistake in the reprint. That should have read intracerebral, and it says intercerebral. In monkeys it appeared possible that it might be a variant. "It" means the mixed virus, the MV strain of
Flexner's might be a variant that lacked the property of multiplication in non-nervous tissues. This also is important because of the decades of work with this strain as the prototype of poliomyelitis virus in man. Of course this was the thing that I have been struggling with and at the end of my stay at the Rockefeller Institute. A specimen of this mixed virus that had been frozen for many years was found to have had no scitopathogenic effect on epithelial cells derived from monkey kidney in any dilution tested—the reason for that is to look for a zone phenomenon in the case of the mixed population that you, of virus particles that you had picked up.

Although intracerebral inoculation of this stored specimen that had been frozen, produced typical paralytic poliomyelitis in the monkey. Now the freshly passaged virus obtained from this monkey also produced no scitopathogenic effect on monkey kidney epithelial cells and four blind passages failed.

Blind passages, that is in epithelial cell monkey kidney epithelial cell cultures—failed to yield a scitopathogenic agent. Although in the third and fourth passages a few epithelial cells degenerated and fell off the glass within four days after inoculation. Whereas the bulk of the growth remained unaffected for ten to twelve days. It should be noted for your, Saul Benison's benefit that the significance of that is that you get a scitopathic effect with a high concentration of virus within 24 hours and it if the concentration is low, you didn't get it. In four days and certainly minute amounts will appear at 70. But this is ten to twelve days, and nothing happened.
Then I say here last year, I observed, that would be 1953, that a strain of MEF\textsuperscript{1} suckling mouse adapted poliomyelitis virus and this was the strain that was adapted in Dr. Olitsky's laboratory by Slesinger--

Q And Kosalls

A And Kosalls. Derived this strain, was derived from the viral progeny in a single suckling mouse--this is, I selected this myself in terminal dilution at the end point of an intracerebral titration in suckling mice, exerted little or no scitopathogenic effect on monkey kidney cells. Notice the point of little or no. It doesn't say no. But after an incubation period of seven to twelve days, the majority of the epithelial cells that had been exposed to the virus even in dilutions as high as one to ten million to one to a hundred million of the suckling mouse strain had become resistant to the rapid--not the word rapid--scitopathogenic effect of the parent MEF\textsuperscript{1} strain that means the monkey, the original monkey brain passage virus before it had been submitted to adaptation to suckling mice. Now similar tests then performed after I had noticed that. It was then that I went back to the mixed virus. Similar tests performed with the MV virus revealed that after an incubation period of ten to twelve days in roller tubes the epithelial cells derived from monkey kidney had acquired a limited resistance to the scitopathogenic effect of one hundred TCD\textsuperscript{50} of the homotypic type 2 YSK strain.

And here is what one saw. At two days after challenge the epithelial cells in the control tube at two days as well as
in the tubes that receive very high dilutions of the MV virus. This is from monkey spinal cord. Were completely destroyed. So the ordinary process, two days, everything was gone. The epithelial cells in the tubes inoculated with the lower dilutions of MV virus. That is lower dilutions of spinal cord of the monkey. Showed little or no change in two days. But at four days almost all the cells were similarly destroyed. Now this is a little different from what I observed and previously described with the MEF\(^1\) strain. You see, there are again differences here. Between the MV, which was monkey brain adapted and the MEF\(^1\) which was suckling mouse brain adapted. The epithelial cells in the tubes inoculated then with--but at four days you see there is then a prongation, a temporary inhibition. This limited resistance characterized by the period of delay was not observed when a mixture of the MV, that is non-scitopathogenic and YSK viruses was added to normal epithelial cells. That is at the same time. Nor was this observed when the YSK virus which is scitopathogenic was added one hour or six days after the addition of MV virus. Now the importance of this is as follows.

The fact that this was not observed when the scitopathic virus was added one hour later meant that some molecular, biological event had to take place as a result of the reaction between the MV virus or the MEF\(^1\) virus with the cell that took more than hour. Because if you added the scitopathic virus one hour later it didn't happen, you see. So obviously this shows that some event that interfered with the normal proliferation
of virus had occurred and required more than an hour. The fact that it did not occur six days when the scitopathic virus was added six days after the MV virus means that that transitory change, biological change that in the first that it was transitory, that it was not a complete resistance that was imparted onto that cell. So these are important observations by themselves indicating somewhat the nature of the interference that the strict neuratropic virus exerts on the cells and a non-nervous cell growing in tissue culture. Of course I am not reading all of this from the--

Q No, you interjected.

A I am interpreting in the light of more recent understanding. My final paragraph says, it should be noted here that strict neuratropic variants that have recently been segregated in this laboratory--this was during the course of my many experiments from type 1 and type 3 viruses--have conferred no such resistance on epithelial cells derived from monkey kidney. But it is possible that the challenge viruses may not have been added at the right time. This again shows the very involved biological events that take place in a cell after infection with polio virus. And it also suggests that the strict neuratropes probably go into the cell, that they do not merely occupy a receptors that some step is interfered with. And it may be that not all strict neuratropes have that capacity. Not all strict neuratropes have the biological characteristics that are similar. They are only similar in that they will not produce a scitopathic effect in non-nervous tissue. I go on to say from these
observations that further work on the mechanism of resistance produced by non-scitopathogenic variants of poliomyelitis virus would be of the greatest interest particularly with respect to the possible operation of a phenomenon comparable to that of licogenic pharge (?).

I personally did not pursue that any further. I have finished reading this now. Again, because this is an example of somebody having to make a choice. Make a choice whether I wanted to pursue, go in the direction that I have already set out, the main line of my work or to go off on a tangent and find out what is it that happens in a cell infected by strict neuratrobe, stokey variance (?) of poliomyelitis virus. To the best of my knowledge this observation had never been picked up by the molecular biologists who studied in very great detail. Of course the work of Baltimore on the minutest detail of the biology of replication of polio virus in cells which added very much to our knowledge not so much about polio virus for the control of polio virus as a disease, as a cause of the raising of disease but for basic understanding biologic phenomena. He pursued that very extensively. This was part of his Nobel oration, the description of that work. And yet just what different mutants of variance of polio virus do particularly those who do not multiply to the best of my knowledge, and I have now checked the literature, has never been followed up. And I am sure that it would throw considerable interesting general biological light on the interaction between viruses and
cells at different phases of replication. So much for this.
If you need this--

Q  Dr. Sabin, before you leave that, let me ask a question that almost seems--you have never had any formal training in genetics before undertaking this, this kind of work.

A  Let me answer this as follows. I never had any formal training in genetics except laboratory. You see in Medical School nor was that the period in which the tools for studying the molecular biology of viral replication were available. Nor may I say that I trained myself in fundamental genetics but fundamental--this is Mendellian genetics until years not years later but actually it happened before I must backtrack a little bit because I am not sure. I think we covered it in a previous chapter when by accident during my work at the Rockefeller Institute at Princeton during World War II, I discovered the complete resistance genetic resistance of mice bred out, white mice bred out at the Rockefeller Institute at Princeton to yellow fever virus and then other viruses by comparison to the same original stock of Swiss mice that were bred out at the Rockefeller Institute in New York. And then when after the war was over I went back to study the genetics of this resistance it was then that I trained myself so it isn't correct to say that in 1953, 54 I had no training in genetics because I trained myself. Because my publications with beautiful genetic laws operating in resistance to infection with yellow fever virus and with dengue virus and certain other viruses in the same group forced me to train
myself in genetics so that I had that training by the time that this work was done. It was enough to make me think genetically in terms of the multiplicity of potentials of individual virus particles but this was not a situation that was comparable to here is a, one kind of a pea and here is another kind of a pea and I cross the two and what do I get. What do I get. This I was able to do. I crossed white mice with a 100% resistance to, resistant to yellow fever virus with mice that are 100% susceptible. I study the F₁ generation. I study the back crosses. I study the F₂ generations. I study the mechanism involved. That was a genetic study. But here, this was a different kind. This had nothing to do really with genetics as such in the classical style as you say. It had more to do with the biology, molecular biology and the chemistry of the phenomenon that go into operation when a virus particle hooks up by proper receptors to a susceptible cell and then a whole chain of events can either happen or not happen or be interfered with, depending on the nature of the virus particle. So this is quite another aspect of genetics. It has nothing to do with inheritance and certain characters when virus particles are crossed. Or, as in the case of influenza when two virus particles with different genetic material infect the same cell. It is quite a different story. A pursuit of the observations that I just described further to analyze the mechanism at what stage, what happened, could have yielded an understanding of the steps involved in replication of polio viruses in cells just what it is that some strict neuratropes lack that prevents them from multiplying in a non-nervous cells and what it is
that they nevertheless do in that cell that interferes with the multiplication of those that can multiply in that cell. Well, so much for general biology which I did not pursue in this particular instance because of the over riding priority in my own judgment of the other things that I had decided to pursue. Now there is—it is an important principle if I may bring this out—

Q Oh, please.
A In the life of a scientist. Because subsequently as a so-called administrator especially, I ran into this the wife's penance to his science (?) and I had to think about this problem. And even now because in a scientist's life there are many occasions when you can ask more questions than you can handle. And there are many scientists who simultaneously try to work on ten, twenty different things at the same time. The same ones. Alright they may have a lot of hands doing many other things. And this by and large is not productive. When I analyze the work of people who tried to spread themselves all over the place they accumulate a lot of pebbles but as a rule—not always—I am saying as a rule they do not end up with any meaningful structure, with any meaningful, larger contribution. And the reason for that is that they had not made up their mind out of the ten or twenty burning desires that they have, which one they should concentrate on. And this is a good example of in my own case, I am also guilty of having tried my hand at many things at the same time. I am not trying to throw stones at
others. But this is a constant problem with me, and in this case I made a decision that, not to divert myself more than is absolutely necessary to get to the answer of the problem that I originally defined.

Q You see me turning pages and what I was turning for was to find something I had marked down and it bears on what you have just said. You found a variant that had an uncogenic effect on helocells and I wondered why you didn't pursue that. It looked like an interesting--

A Saul--there is something wrong in this statement a priori. I did not find a variant that was uncogenic for helocells because helocells are malignant cells.

Q Yes.

A So it couldn't be a variant. I don't know what you have in mind.

Q Let me shut this a minute.

Dr. Sabin, in your semi annual report to the National Foundation for the period of January 1, 1955 to June 30, 1955 on page 4 at the bottom of the page under item number 5 you have said the following. Many unclassified viruses have been recovered from the stools of chimpanzees during the course of our poliomyelitis studies. One of these strains was sent to Dr. Huthner (Huebner) who was looking for non-human viruses to use for experimental therapy of human carcinoma of the uterus. This chimpanzee virus while scitopatogenic for helocells and human sera contained no antibodies for it. In preliminary tests it produced extensive destruction in uterine
cancers. The point of this observation is that a battery of antigenically distinct chimpanzee enteric viruses for which human beings would have no antibody might lend themselves to consecutive administration in tests for their destructive capacity in certain human cancers.

And I wonder if you would comment on it because I think it bears on what we have been talking about.

A I would like to comment on this because it represented a very important effort during that period in which an attempt was made to use viruses to destroy human cancers. Should I continue on this--

Q Please, please. We have plenty of tape.

A Alright. Now here is the background of this. When tissue cultures came into use for the isolation of viruses and it opened up Pandora's box progress, one of the cell lines that was used very commonly because it was easy was used for polio as well as other viruses was the helocell. The helocell was regarded at that time as having been derived from a carcinoma of the cervix of the uterus. Cervix of the uterus and perpetuated. Now, in the study that subsequently followed in various laboratories and particularly Dr. Huebner and the National Institutes of Health pursued this. It was found that many of the so-called echo and coccascki viruses that were being isolated from human stools had an effect not only on helocells but on other cell lines derived from human cancers. There are not many. But, so the question naturally arose, would it be possible to use such viruses in human beings--
viruses that would be, not be harmful to the human being to destroy a cancer in the living animal. If these relatively innocuous orphan viruses as they were called at the time, could destroy cancer cells in vitro much better than any drug that you could find, why not use them in the human body to destroy cancers. Some experiments had been done in model systems in which it was indeed found that this could be done. I myself carried out an experiment which was reported in the literature. I don't know whether we discussed it earlier with Alice Moore.

Q Hold it. Hold it. Go ahead.

A Of the Sloan Kettering Institute. It had been found that a transplantable sarcoma of mice—the sarcoma 180 was very readily destroyed by Russian spring summer encephalitis virus. So that if you had a mouse that carried this tumor and you inoculated it with Russian spring summer encephalitis virus that localized in the tumor, it destroyed the tumor but it also killed the mouse. So you didn't know what was going to happen. But then I found, and this relates to our previous discussion, that when we used the Princeton Institute mice, that were genetically resistant to yellow fever virus and to certain other viruses which included Russian spring summer encephalitis group, it was possible to inoculate the virus and the mice would not die. So the question arose, what would happen in a mouse, a Princeton Rockefeller Institute mouse, a resistant mouse that would not die of Russian spring summer encephalitis but which was carrying a big tumor of sarcoma, a transplantable tumor sarcoma 180. Dr. Alice Moore at the
Sloan Kettering Institute was working with this. So we transplanted some sarcoma 180 into Princeton Institute white mice; they developed big tumors. And they were inoculated with large doses from varying doses of Russian spring summer encephalitis. Well, to make a long story short, the virus dissolved the tumor. It did. Because the tumor was genetically different from the mouse, even though it grew in it. It was well adapted to growth. And the virus multiplied to a very high level in that tumor and destroyed most of it. The mouse survived. So that was in an era, you see, where this was under considerable discussion and there were some seminars devoted to it. One of the problems was that if you used viruses that were commonly disseminated among human beings that you would not be able to find human— that the vast majority of human beings had immunity to them, and that it wouldn't multiply in them. Now you can't use a virus like Russian spring summer, it would kill them, you see. There were some other experiments tried with Wes Nile for example, so when I run across in the stools of chimpanzees a virus that kills helocells in tissue culture and for which as I said, human beings have no antibodies, I say why isn't this a wonderful opportunity. Here we have a virus that will destroy these cells and we have no problem of immunity in human beings. And that is why I turned it over to Huebner who by that time after many studies in model systems in mice had already begun to collaborate with surgeons who did a very wonderful thing. In order to prevent too much of the virus being distributed throughout the body before it got to the tumor itself, they would isolate the
arterial supply to the tumor and actually inject the virus into the artery supplying the tumor and they had found already with some other viruses in patients who had no immunity that this would indeed punch a hole in the tumor. It would eat away much of the tumor. Pretty much the way certain chemotherapy would do. But the problem was, that no sooner than that hole was punched, and as the individual developed immunity to that virus, the tumor cells began to grow again, and after a couple of months you were no further ahead. A situation not unlike that with chemotherapy. So, during a symposium that was held during that period at the M. D. Anderson I proposed the following strategy. I always was influenced. This is an aside. By my training at Carlisle Barracks as a medical officer. I didn't get much basic military strategy but it had an impact on me because there was very much in that strategy that has to do with an attack on human disease. So I thought that the following strategy may be worthwhile.

That if one virus will destroy a large number of tumor cells in an existing tumor and then it stops why not come in with a second blow. In other words, you have the first group of troops advancing, just opening up a hole and then before that hole can be filled in by the enemy, come in with another one and destroy the others. And with a third one and with a fourth one. So the suggestion that I made was that one should not give up this work simply because the body becomes immune and you cannot destroy the tumor completely. The strategy that I proposed is that we should develop a battery
of viruses. Viruses that would be non pathogenic for man, and when you are dealing with patients who are going to die of cancer anyway, the problem is not so great. And just give them one after another. After one knocked out a part of a tumor, individual is immune. Give them a second one. Knock out some more. Give him a third one. Knock out some more. Actually years later, this became the approach of chemotherapy, you see. But, this was not pursued. So when I say in this report that Dr. Huebner reported to me that this virus that I casually picked up, or accidentally picked up from the stools of chimpanzees did produce an effect in destroying uterine carcinoma. Actually it is cervical carcinoma, I felt that it was not pursued far enough by the virologists who took this approach. They left the field to chemotherapy. And I am not at all sure now more than twenty years later that that approach isn't at least as good as the approach that is being made by chemotheraphists against cancer. And may indeed provide additional weapons but it does require the development of a battery of viruses which can be given one after another with a destructive effect on a particular tumor cell. Period.

Q Not period. I have--
A I want to say here--
Q Okay. Well I find interesting the problem is interesting. You are not seduced by it.
A Well I am not seduced by it because we are back to where I started. The decision whether an individual scientist has to make who is interested in carrying his work to the
meaningful end point, to an end point of decision that if I would suddenly drop everything else and go off and pursue this particular thing which at that time was already being done by Huebner and others, that I probably would lose the imput that I had already made on a major objective. The point was that if a scientist works all sorts of interesting observations arise on the side. Sometimes they are more important than the original objective. My judgment was that my original objective was more important than this. And that I had to concentrate the efforts on the original objective until a yes or a no answer would be available. Mind you this is 19---the work was done in 1954. I was still a long way from knowing which way it would go. But I am very glad I made the decision. Although, I just finished saying that I think that that work was left prematurely, that it was still desirable because of our lack of knowledge of how to prevent cancer to use in addition to chemotherapy to use what I would call viral therapy of cancers, viral therapy of cancers and selecting viruses particularly the many orphan viruses from closely related primates that would have a destructive effect on cancer cells in vitro---using them one after another. This is a variation of the strategy. Now this is not without difficulties because unless you carried out very careful studies beforehand you might run into a virus that might be very pathogenic for man. And this is one of the reasons that the work really again was left. Of course some of the chemotherapeutic agents they are using now are pretty damned awful. I doubt
whether there are many virus diseases that can produce the serious side effects as the chemical agents.

Q Now period. Dr. Sabin at this point I would like for you to read into the record a letter of October 3, 1955 which is a summary letter. And I think an important letter.

A Actually it is a supplementary letter. It is addressed to Dr. Koom of the NFIP in which I say that in accord with your request I am sending you herewith a brief progress report to supplement the semi annual progress report and the information contained in my letter of September 10 all this for presentation to the committee on virus research and epidemiology. The first topic is the search for strains possessing minimal pathogeniticity for monkeys by the spinal route.

Since the semi annual report was written three such strains turned up during our screening of poliomyelitis viruses recovered from the stools of healthy children. I should add which we ourselves recovered from the stools of healthy children. The stools having been obtained from Drs. Fox and Gelfain in the New Orleans region. This included two type 1 strains, one designated P-2149 and the other P-2226 and one type 2 strain designated P-712, Seven, twelve. That is seven twelve. It is just one. Now the search for variants capable of producing the scitopathogenic effect in capuchin or capuchin monkey kidney cells revealed that some, but not all type 1 strains may possess a few particles out of a very large population which can produce a scitopathogenic effect in capuchin monkey kidney cells. These particles have been segregated from the others and passaged in
a series, in series in capuchin monkey kidney sufficiently to get away from the original population of virus particles derived from cynamologous monkey kidney cultures. Although tests on several strains are still in progress those that were completed on one type 1 strain indicated that the particles which have the special capacity for producing the scitopathic effect in capuchin cells are as pathogenic for monkeys by the spinal route as was the parent virus.

Let me digress here. This is an example of the attempt to pursue every possible selected medium that might provide polio virus particles with different properties. The ideal properties we were seeking. And this is the main reason for having pursued this. And this is an example of population genetics of virus particles, polio virus particles population genetics is quite different from the genetics of individuals. Alright. I go on and say although tests on two other strains are scheduled for the near future it would now appear that this approach is not promising and will therefore not be pursued further in 1956. It had to be done. It was done, and out. Further work in progress on attempts to find variants which will propagate in chick embryo indicates that this is not a promising line of work and will not be pursued in 1956.

Additional observations on human volunteers. Most of the men who were fed the experimentally segregated attenuated poliomyelitis viruses in January 1955 were available for a check on the level of their antibody six months after ingestion of the virus. As an aside I want to say here that this was refers to work we have not yet discussed in detail. This refers
to the work on human volunteers in the Chillicothe Federal Prison.

Now I continue from the letter. In all instances the titers were either the same. That is the titers of antibody, six months after ingestion of the virus were either the same or higher at six months than at three months. In a number of instances the higher titers occurred in those volunteers who were fed a second type of virus three months after the initial ingestion of another titer. As an aside I would like to say here this is an important observation that was followed up later which indicated that you can get a booster virus against--not a booster virus--a boost of antibody titer when you are infected not by the same type but by another type. In other words that the cross relationship between the three distinct types of polio virus is such that you may get a temporary boost let us say in type 1 antibody even though the infection was with type 2. And this is still until this day not understood because the assumption is that in an unknown patient if he has had a--he shows a boost, a rise in antibody for type 1 he had an infection of type 1. It is terribly important and it became important subsequently during the mass trials when one tried to determine the nature of intercurrent paralytic disease to decide what a rise in antibody meant. Did it mean that that person was infected in nature with that particular type. It doesn't. That is what this work showed, you see. I am going back to this.

Q Go ahead.

A In my letter of September 10, I wrote you of a new series--excuse me again for stopping here. I keep speaking of my letter of September 10, and yet I don't have a letter
of September 10. I read into the record a letter of June 25, 1955 but nothing. I have no copy of a letter of September 10, and I don't know what I said in my letter of September 10.

Q Alright.
A Would you stop this a moment?
Q Yes, yes.
A We have been unable to locate the letter of September 10 so I will just continue reading from the letter of October 3. And this paragraph, this paragraph begins in my letter of September 10, I wrote you of a new series of tests which were to begin at Chillicothe on September 13. A total of 27 men were used. Six men were used for each of the three type 1 strains that were tested. That is LSC P-2149, and P-2226. Six men for the type 3Glen strain. Parenthetically this is a strain isolated from a healthy child in Cincinnati that was relatively avirulent to begin with. And three men who lacked both type 1 and 3 antibody received a mixture of equal amounts of type 1, P-2149 and type 3 Glen viruses. Each strain was tested in a dose of 0.1 ml and 0. and one thousandth of an ml. The viruses this time were fed in a cherry syrup rather than in milk in the hopes that this might lead to a larger number of virus particles being trapped on the throat. It is interesting. From the results that have been obtained, it would appear that this was achieved although one cannot be certain to what extent the strain of virus might have played a part. At any rate while none of the volunteers who received virus in milk in January from a dose of 1 thousandth ml had
virus multiplication in their throat. Six out of eleven of the present group presented evidence of viral multiplication in the throat after ingestion of one thousandth ml. Alimentary infection was established in all volunteers in the test performed on serial bleedings up to two weeks after ingestion of the virus indicated that all but one developed antibody. Many of them within one week after ingestion of the virus. The one who thus far has not developed antibodies received one tenth ml of the LSC virus and has exhibited very active viral multiplication in his throat and lower alimentary tract.

Let me digress here. This is one of those many observations that I encountered in several hundred volunteers I studied who didn't follow the classical concept. The classical concept is that polio virus multiplies; antibody appears within about a week, etc., etc. That is classical alright because that is the way it usually happens but there are these exceptions that we must remember. And here is a beautiful one which we encountered again later of where the dose of virus that is fed is big, active viral multiplication goes on in the throat and lower alimentary tract, but he had not developed antibodies. So the mere absence of antibody is by itself no proof in any one person statistically it may be significant. But in any one person it doesn't mean that he didn't have a previous infection with the virus. That is determined only by his response to subsequent infection.

Now I continue reading from the letter now. Many of the volunteers, particularly in the group who received I say here
who received P-2149 virus developed an antibody within one week after ingestion of the virus. Of special interest is the fact that all three volunteers who were fed a mixture of about one millionth TCD$^{50}$ of the type 1 P-2149 strain and the type 3 Glen strain developed antibodies for both type 1 and type 3 strains. This lack of interference in the case of these two strains in human volunteers is to be contrasted with the complete suppression of the multiplication and antibody response to the type 3 Leon virus when it was fed to chimpanzees with a mixture of our Mahoney and YSK attenuated viruses. That is with type 1 and type 2. Preliminary tests on the current human volunteers already indicate that apparently both type 1 and type 3 viruses multiply simultaneously in the pharyngeal wall. However, many additional tests still remain to be done before a complete picture will be available.

I want to digress here a moment, because these statements in this letter here are a reflection of the numerable facets that had to be elucidated. The numerable questions which are just as basic as any other questions would have to be asked as to what would happen if you would feed these strains separately, if you feed two together, if you feed the three types together. Are there strains which may interfere less with one another--all of that work had to be done in advance of any decision of rushing out and administering the first strain of virus that you had that looked like a plausible candidate. When I read back on this now I am amazed at the tremendous amount of preliminary
infrastructure studies that I believed were necessary that we carried out and that provided us ultimately with a spectrum of knowledge about the behavior of polio virus strains in the human alimentary tract that is still up to the present time not properly perceived by my colleagues who have not done all of this work because I have never written a monograph in which I documented every one of the experiences that have left such a deep impression on my own thinking.

Q Now since you have raised this question of infrastructure of research--

A Of knowledge.

Q Knowledge. For example, by the end of 1953, beginning of 1954 you have several strains which are avirulent on spinal inoculation in chimpanzees. Why do you continue research for a variant that is also avirulent for monkeys?

A I think this question was touched upon before and the answer is simple. The striving was to determine whether any strain we possessed or anybody else possessed could be as non-neurotropic for the most highly sensitive primate and still multiply in the intestinal tract enough to produce (a) the systemic immunity that we were measuring and looking for as well as the resistance to reinfection that is an essential component of the whole challenge of breaking the chain of transmittance. We did not want to give up. It would be very easy to say alright, this is good enough. But it was necessary to continue. You have to have here a judgment how long do you continue, and when do you stop. And I would say the judgment
depends on the results that you gain from a certain effort. If you continue too long and you don't do anything. That's not very wise because you may never get it. You might get it. As a matter of fact, I continued. I did continue as I think I remarked before after the worldwide tests on increasingly larger numbers of human beings were already in progress. I did continue after the vaccine had already been administered to over 100 million people in the world. I did continue with the search by cold adaptation, by getting viruses that would multiply at 25° which they ordinarily don't. And did succeed in obtaining mutants that even on spinal inoculation in monkeys were not active, that produced practically--were not paralytogenic. In other words, I achieved that. And then I continued with the experiments in chimpanzees and finally feeding to children and they turned out to be much worse from the point of view of quick reversion to larger numbers with increased virulence in the human intestinal tract that we had selected. So, we did achieve finally strains that were inactive on intraspinal inoculation in the monkey but they were no good. For other reasons. Okay.

Q Go ahead.

A Now, we go back to the letter of October 3, 1955. And the next, the final, the beginning of the last paragraph on page 2. I say tomorrow on October 4, a new series of tests are scheduled to begin on another group of 20 volunteers at Chillicothe.

Let me digress here. The numbers of volunteers that could be used at any one time in Chillicothe were influenced by several factors. We were dealing there with young adults.
Some of them had immunity already naturally acquired immunity to all three types of polio. Some lacked immunity for one or at least antibody for one or another type. So we had to constantly screen new numbers because there were, I forget, a couple of thousand young prisoners in this federal prison. We had to screen them, bleed them ahead of time, find out those that were without antibody for the type or types that we were going to test. And then there was another constraint. The warden of the prison said all of these prisoners are working and you cannot disrupt my various activities in the prison by taking out too many people. You can have so many at a time. Alright. So that is the reason for some of the numbers that we used.

So another test was to begin on another group of 20 volunteers at Chillicothe. Twelve men will be used for the type 2 strain, P-712 in doses ranging from one tenth to one thousandth of an ml. This is the standard, preliminary. One man who is without antibody for all three types of polio—they were rare as hen's teeth in that age group and in that social group—will receive a mixture of all three types. Four men who are without antibody for type 1 and type 2 are receiving a mixture of 1 and 2. And three men without antibody for types 2 and 3 will receive a mixture of types 2 and 3 virus. The studies on both groups of these volunteers should be completed by the end of this year, and at the end of that time we may be in a position to decide which of the strains are most suitable for further progressive studies in human
beings. An emphasis on the step by step approach. I continue from the letter. At this time we are testing in monkeys. Always the first step, monkeys, chimpanzees, humans. At this time we are testing in monkeys two additional type 3 strains which were recovered from healthy children in the New Orleans area.

Q This was Fox and Gelfain.

A Yes. Only if they should exhibit a markedly lower pathogenicity for monkeys by the spinal route than the Glen strain. That is the Cincinnati strain from---that's the strain from Cincinnati children. Now under investigation would tests in human beings be carried out.

I will digress again. This is an illustration of the indicators for decision. At this stage if it was not better intraspinally than others we had already studied, it stopped at the phase of tests in the monkeys for neuralvirulence. If it had been better we would have gone on to the next phase. Now I continue from the letter. As matters stand now, therefore, it would appear that the major effort in 1956. Let me digress again. This shows the constant job of planning ahead and trying to project to a group who has to provide you with the funds and so forth. And it is a necessity. I am not speaking against it---but of being able to project ahead what you are likely to do on the basis of what you know now. What you ultimately do may turn out to be altogether different because you don't know what the results will dictate. So that is why I say as matters stand now therefore it would appear that the
major effort in 1956 would be concerned with an extension of studies in human beings of the strains that proved to be the optimum ones in the current tests. These studies would be essentially the ones that were outlined the memorandum accompanying the 1956 application. It would be my plan to prepare at least ten liters of each of the selected strain, carry out the most extensive safety and control tests on them, and then use aliquots of these basic lots for all the projected studies in human beings.

I am digressing here once again, because this has the important principle. It touches on the important principle of starting with a seed lot, a lot big enough so that the variation from lot to lot as it grows will be eliminated; this is an attempt to eliminate as many variables as possible. But you are not ready for that sort of thing until you have a potential candidate.

Q  Alright. Hold it. We are running to the end of the tape.

END OF TAPE.