Q With a detailed step by step, could you summarize the early results of your work at Chillicothe. Let us say from '55 to the middle of '56.

A Well, in my initial request for permission to work there from the director, medical director of the bureau of prisons I said I would rather have at least sixty volunteers that I can use. The proper immunologic status, and I thought that it would be a matter of months and so we would be carrying but actually as it turned out ultimately. I forget exactly now the work continued '55, '56 and a good bit of '57. By the middle of 1956 when it appeared to Dr. Janney the medical director of the bureau of prisons that I seemed to be going on and on he was pressuring me—I wouldn't say pressuring me—but he wanted to know when he would have a report. And here is a letter of August 27, 1956 and I might read it in its entirety and comment as I go along because it reflects two things. It reflects my constant state which still hasn't disappeared of having to do, go here go there not always—with some report. Some need hanging over my head, and secondly it summarized the situation. The letter goes like this. Dear Dr. Janney. To begin with I must apologize most humbly for my neglect in complying earlier with your request for a brief summary of the polio vaccine research project that I have been conducting at the Chillicothe Reformatory. For more than a month prior to my departure for Europe and during the few weeks since my return the pressure of business is so great that I fear that I could you a proper report. I hope very much that you will forgive me because I
want you to know how deeply I appreciate your own cooperation as well as that of everyone concerned at the Chillicothe Reformatory. I would like to break here and say why do I go to Europe in the midst of all this study. Later on I hoped there will be an opportunity to discuss the fact that beginning in 1956 when I was invited to go to the Soviet Union during the summer of '56 when a plan of collaboration began I went to the Soviet Union every year as part of a collaborative effort in this field because there were many more opportunities to begin to study problems in the field or in institutions that were quite different from the opportunities that I had in the United States. So part of my trips to Europe was that there was this collaborative effort in the Soviet Union going on. Also at a certain stage a collaborative effort began in Holland and in some other places so it was necessary to--to get away from Cincinnati and this work but part of a larger effort. Now I will continue with the letter.

The main purpose of my research at the Chillicothe Reformatory is to study. I say is because it is still going on. Is to study in great detail the behavior of a variety of attenuated strains. And I want to stress here the word variety. Of attenuated strains of polio virus in human beings with a view to the ultimate selection of the best strains for inclusion in an attenuated polio virus vaccine. This again emphasizes that it was necessary to carry out studies in human beings for the ultimate selection but it was not--that we were doing now in human beings studies with strains that we said
these are the best. No. We also had to use studies in human beings to help in our decision. That is the point of this.
From January 1955, that is when I began, up to the present I have carried out studies on 116 inmates of the Chillicothe Reformatory and four of the doctors serving at the Reformatory, that is Drs. Hackett, McCabe, Lap, and Dixon. Many of these men were used in several tests. The first series of tests on 30 men was carried out in January 1955. As an aside I will say here right after I carried out a serologic survey to find out whom I could even ask to volunteer.

In April 1955 17 of these thirty men that I did--that were started in January were used over again in another series of tests. In September and October 1955, another group of 48 men including five from the previous group who were used over again, participated in a series of tests with new attenuated strains. What, an aside here. While the work was going on in human beings and we were learning certain things, the behavior of a strain, a given strain or set of strains, in the humans, work was going on in the laboratory with monkeys and chimpanzees so that it was a feedback mechanism. Alright. I will continue.

In April 1956 47 men were used in tests with the three optimum strains selected from those previously studied. Now we are now into the following year. This is April 1956, you see. In April 1956 47 men. This is in addition to those mentioned before. Were used in tests with the three optimum strains selected from those previously studied which
were grown in a large quantity for the first time by a commercial company. Included in this group were also nine men who had been given two doses of Salk vaccine prior to the administration of the attenuated virus by mouth as part of a study to determine whether the two can be used together. No significant illness occurred in any of the volunteers. When some of the naturally occurring attenuated strains were fed a certain proportion of the men had a slight transitory sore throat, and occasionally headache for a day or two. These are the naturally occurring attenuated viruses. We continue with the letter. Although a tremendous amount of new information about the multiplication and behavior of attenuated polio viruses has been obtained as a result of these studies I should like merely to mention the following main points which I regard as of the greatest importance in orienting the progress of the work on an attenuated polio virus vaccine. (1) the discovery that the human intestinal tract is much more sensitive than that of the chimpanzee as regards the capacity of attenuated strains to multiply in the intestinal necrosa with the production of an immune response. This showed that the ultimate efficacy of an attenuated strain can be established only by tests on human beings because we have found that the intestinal tract of monkeys was even more resistant than that of chimpanzees. (2). Before I go on to two I should like to say that this is a subject we've discussed before but here it was obtained on larger numbers in a quantitative way which we couldn't do before. When I said we knew that before. We knew of the difference between monkeys and chimpanzees but it was not until
we got into the human beings that we could learn the difference between chimpanzees and humans. So that is the importance of this discovery. These are discoveries. This is—I don't know if you know. I must again digress here. I am constantly disturbed by the lack of understanding among certain scientists who use the words basic and applied research. Is this applied research? Is this basic research? I think there is a misconception. I think that whenever you search for understanding of a phenomenon whether or not it be directed towards the achievement of some other specific objective. That is basic fundamental research. This is, in my judgement, fundamental, basic, categorical research. Categorical in the sense that it is being done in order to be able to achieve something that could be used for the control of the disease. So it is categorical. But it is still fundamental. You have to learn first of all about the underlying biological phenomenon without which you can do nothing. Alright so much for an aside because I am sensing—

Q No, I think that kind of aside is—

A Point number 2. Because of this greater sensitivity of the human intestinal tract it is not possible to produce a closed infection in human beings by parental injection of attenuated strains since even the small amounts of virus which may be absorbed into the circulation from the parental site can localize in the intestinal tract and multiply there with excretion of virus in the stools. Let me expand on this. You may recall before that we really didn't know whether if
the vaccine were to be given intramuscularly instead of given by mouth you could have a situation in which it would multiply as yellow fever does, at the site of injection. Let's say the regional lymph nodes and somewhere else. Give rise to an immunity and it would be a closed thing. It wouldn't spread to others, which could have its advantages. You had to learn. But what did we find, according to this. We found that in volunteers who received the virus intramuscularly that it localized in the intestinal tract anyway and multiplied in the intestinal tract so you didn't have a so called closed infection when you gave polio virus, even when you gave it not by mouth but by injection. So this was a fundamental discovery which you couldn't predict. And furthermore you couldn't tell by using monkeys where it doesn't multiply. Where even in virulent virus doesn't multiply enough that we detect it in the intestinal tract and--alright let me go on now with point number 3.

A strain of virus which cannot multiply in the body after intramuscular injection except when big enough doses are used to permit secondary localization in the intestinal tract can nevertheless readily multiply and produce an immunogenic infection when small doses are given by mouth. This is a basic discovery. That you have a differential, and susceptibility of tissues reached by the virus after injection from that in the intestinal tract and this really eliminated for all practical purposes the possibility, the practical possibility of using a live virus, live polio virus by injection. You needed
a lot more and when you used a lot more to get the immunogenic infection it localized in the intestinal tract also. In other words these original orienting studies on human beings eliminated any other route except the oral route especially. A very important point. You couldn't do by theoretical considerations or experimentation on animals.

Fourth point. No evidence of multiplication of virus was found in the mouth, in the gums or tongue when virus was given by mouth. When large doses were fed the virus multiplied in the throat and in the lower alimentary tract and with small doses only lower alimentary tract. Infection of the posterior pharyngeal wall could be produced with regularity when smaller doses were swabbed directly on the mucosa. This again is fundamental work in search of understanding what happens. It is important to know before you go out and give vaccine to thousands and hundreds of thousands and millions what happens. Does it multiply in the mouth. Does it in the gum, in the tongue. We have to know all this. This is an example of a proper study of a vaccine before you go off and you shoot it to everybody. I am sorry. I am saying this was the work that I did. But I think it is an example which is now generally recognized but very often not by those who are proposing new vaccines and certainly it was not done by at least two other investigators who were working on it, for a procedure for vaccination with a live virus vaccine.

Point number 5. Most of the strains tested produced no viremia. That means when given by mouth despite extensive multiplication in the alimentary tract. In the actual data.
I am digressing here. We are not dealing with qualitative expressions like slide, moderate, extensive. We measured. We didn't just test for virus in the stools. We titrated the amount per gram, you see so that this was all the work that was done, so you would have a quantitative understanding. On the other hand. I am continuing from the letter now with some of the naturally occurring attenuated strains traces of virus were found in the blood usually for one or two days.

Point number 6. Considerable variation was encountered in the neuratropism of a virus recovered from the stools of the volunteers when different strains were fed. And this provided a basis for selecting some strains and discarding others. I want to digress here. You couldn't do that on chimpanzees. The chimpanzee is screen number one. And why. Because the number of mutants of variants that arise and this is population genetics is proportional to the number of generations and to the level of viral multiplication. If the level of viral multiplication is low the probability of certain mutants arising is very low, very small. And the more multiplication the more is there a probability of variants to emerge. And that could be done only in humans. Because even when you had multiplication in the intestinal mucosa of chimpanzees it was much less. It would be a hundred to ten thousand times less than in the intestinal mucosa of human beings. This was very important to understand why if you are going to try to develop something that has to be used in human beings there is no alternative ultimately to studying it in human beings. People especially in this era who are rightfully
concerned with the ethics of human experimentation I think must understand this sort of thing. This of course does not absolve an investigator from very carefully justifying just this sort of thing. Whether or not that which he proposes to do in human beings (a) is necessary and (b) cannot be acquired. That knowledge cannot be acquired in any other way. I am going to continue again from the letter.

It should be noted however that even with strains of virus that were discarded because of the number of mutants that were produced during multiplication in the human alimentary tract, the increase in neurotropism of the virus recovered from the stools was not sufficiently great to produce paralysis in chimpanzees inoculated directly into the spinal cord with about a million tissue culture doses of virus. Let me make an aside here. We never dealt with a problem of reversion to virulence, to original virulence. And this again is still not understood even by some very good virologists whose understanding of the genetics, the population genetics of a viral culture or of the mutations that occur in the factors of selection that we are dealing here with quantitative step wise things. Sure, professional bacterial geneticists, professional geneticists understand this. But many of the so called infectious disease people or virologists who are not involved in such studies, they think very simply. Does it revert. Does it not revert. That is not the way things happen. The question is how far does it go. And we had to look for the optimum. And that is why the importance of this statement, that even the ones that we discarded because there
was a greater increase above that which we fed, it was still not sufficiently great—the increase in neural virulence to be pathogenic, paralytogenic in chimpanzees when about a million doses were put directly in the spinal cord. So I think this whole concept of reversion to virulence which is rightfully important. A problem that one must very carefully consider and continue to be considered particularly even later in human field trials. It was a tremendously important point that had to be resolved because this was a totally new kind of live virus vaccine. It was not like yellow fever. It was not like any other because here was a vaccine that was given and it was expected to replace the naturally occurring paralytogenic strains. It would have to be. It would spread in a population. Therefore the knowledge that was necessary to know what would happen if it spread. Would it achieve the kind of virulence that—well the world was full of it anyway. This is what we were counting—in other words it wouldn't create a new monster. It couldn't. But, what would happen if it continued to spread? This was information we needed absolutely. And I can tell you it occurred again and again in local scientific debate, in international congresses, in public health considerations because this is a new and revolutionary kind of vaccine. I think people still do not realize. They think it is just another live virus vaccine. It is not. It is a totally different thing because for the first time in the history of medicine you are replacing a potentially—we didn't know then that we would—now we know that we have achieved this. We are replacing what has occurred in nature over eons of years in association
with the human race by something which we have created artificially. That is not true of small pox vaccine. Small pox vaccine provides immunity. It does not create another virus in nature which perpetuates itself and continues to spread in the population. It is not true of yellow fever. It is not true of measles. It is not true of any live virus vaccine, and this is why people still do not realize. They are naive. I speak with feeling on this because I am really amazed at the naivete of some of my sophisticated colleagues. Alright. Now that I have worked off steam I am going to continue to read from my summary to Dr. Janney.

Q Go ahead. This is wonderful.

A The reason for this is that, as I read this summary now and it is exactly almost twenty years later, in the light of what I have gone through and I find that something that was discovered here by a very meticulous, methodical studies of what you might call comparative virology. Studies in primates of different stages of development still is not appreciated. Is this basic research or is this applied research off the shelf. Well okay. Continued. I have just talked about the fact of so called reversion, so called important role of mutants that arise. Now point number 7. No evidence was obtained of spread of virus among the volunteers. Now I must say an aside here. I don't know what the rest of the paragraph says but with some of these volunteers without immunity to polio the amount of virus per gram was as much as one million infective doses and sometimes more per gram. It wasn't the same in every person.
and that is why the study of larger numbers was helpful. And therefore and I wouldn't say that their hygiene was particularly good except that they were part of the culture in which they did wash their hands I presume, some of them after they went to the toilet, and they had as young adults aged 21 to let's say about 30, habits which small children in the richest, and the best homes do not have. Small children have their hands contaminated with feces when after they are left on their own to wipe their little behinds, you see. In any kind of culture. So that what we learned in the adults was merely what applied to this kind of an age group, and this kind of a pattern we had to learn later. So, there was--no evidence was obtained of spread of virus among the volunteers. Now if we had only the volunteers. I am still reading, not reading from the letter, we would have an erroneous impression. We would say oh well, alright, it multiplies in the intestinal tract but it doesn't spread. In effect when we began to study things in the field under different conditions we found that it did spread. And it was a darn good thing that it did spread. So now I am going to go back to the letter.

Thus although all tests involved groups of volunteers which were fed either type 1 or type 2 or type 3 virus we never recovered a type of virus from the stools of any volunteer which he had not been fed. Furthermore, a special test carried out on a group of five intimate associates of a volunteer who was excreting maximum amounts of virus showed no evidence of infection among them. Also no evidence of transmission of infection was found in the home of one of
the doctors who had a wife and young child without antibody to the virus which he was excreting. Now, again I want to digress. I want to say that obviously in order to be able to do a meaningful test on transmission we selected in order to do that special test to have intimate associates we had to have intimate associates without immunity to this type otherwise it would mean nothing. So you see it was necessary to always to have a known pool from whom to select. Even so, as we found later the greater multiplication of the virus in young children, the greater susceptibility of the intestinal tract to infection with small amounts was a factor that very fortunately contributed to dissemination.

Point number 8. It was found that in certain individuals with certain strains it was possible to obtain concurrent infection with two and even all three types of polio virus when they were fed simultaneously. It was also found that the three types of virus could be fed seriatum, at three week intervals without interference. This is again an aside a much larger subject about which much more could be learned and the crucial point at that stage of the game, the crucial words here are in certain individual with certain strains because as it ultimately turned out sure you could feed all three types at the same time but you wouldn't get an adequate immunity to all three types in the vast majority. Now my last point.

Number nine. Antibody produced by two doses of Salk vaccine. I want to have an aside here. Obviously volunteers
were selected who had no antibody at all before they got it because what we were trying to do was to determine what two doses of Salk vaccine which was the thing that was already being in 1956 already being widely used in the United States what how would that affect subsequent infection with let's say these attenuated strains. So we took volunteers who had no antibody. We gave them two doses of Salk vaccine. See what kind of response we would get and then fed them. So, now I read from the letter.

Antibody produced by two doses of Salk vaccine did not interfere with multiplication of the virus in the alimentary tract either as regards to the amount of virus excreted or the duration of virus excreted. This was a marked contrast to the results obtained in studies on individuals who had naturally acquired antibody or who had acquired such antibody after the feeding of attenuated strains. This again, now I am not reading from the letter was a fundamental discovery that was that actually destined to play a most important role in the strategy of using a live virus vaccine within a population that had Salk vaccine. And furthermore it was a fundamental discovery that showed that by and large with certain rare exceptions the antibody produced by Salk vaccine which could protect an individual or a certain proportion of individuals from development of paralysis was incapable of stopping the chain of transmission of polio viruses and under such conditions epidemics would continue to occur. Not as great but infection you would not be able to control the natural history of poliomyelitis. You would only be able to control
the response of a certain proportion of individuals.

Q You know that probably is one of the most interesting things that you have just said did anyone pick this up, let us say at the Foundation when you sent it.

A Well it was not only picked up at the Foundation it became a subject of a great deal of work. Because subsequently three doses were used, then four doses were used and Dr. Klugman and I carried out a collaborative study at Willowbrook State Hospital after a certain chain of events in which we took young children who had no antibody at all to begin with. We gave them four doses, spread at the optimum intervals and then fed them virus and discovered that even under those conditions it had not influenced the multiplication. On the other hand there is a continuing controversy going on particularly with. Of course Salk would never admit that but I mean the evidence was there. Sven Dahl also carried out a continuing thing and he said that when we administer our very good vaccine that we killed vaccine that we make in Sweden and we give multiple doses and they develop very high levels of antibody that multiplication is interfered with. Well the fact of the matter was that whether or not there is a certain amount of interference because we studied that subsequently in the majority of instances that didn't occur. And the thing that finally won out was not the arguments pro and con among different people virologists experts in the field but such experiences that occurred repeatedly in communities in the United States and abroad a city like Syracuse after they had
carried out extensive immunization with Salk vaccine. As extensive as possible. Three doses, four doses in the majority along came a severe epidemic of paralytic polio and we had to step in at that time we already had the strains and use live virus vaccine to stop. The same thing happened in Atlanta. The same thing happened in Italy. The same thing happened in Czechoslovakia in 1961 within a couple of months after something like 14 million children had received three doses, two of the three doses of Salk vaccine there broke out the most severe epidemic that they ever had in the history and presented other interesting public situations you see you talk about sociology and politics will enter into this field too you will see, in a moment. So that the evidence in the field was that extensive use of Salk vaccine did not prevent dissemination and did not prevent epidemics which might have been bigger, it might. Instead of having a thousand you might have had two thousand cases. Instead of having two thousand cases you might have had one thousand or eight hundred. But it did not prevent the occurrence of epidemics. And I think the situation which is still going is the discussion even now twenty years later why is it that in Sweden their live virus vaccine is not used or in Holland which are two places in Europe. Why is it that they are not used. My answer to that is because all of the countries around them, you see, have used live virus vaccine. The transmission of the paralytic strains has been interrupted and so they are being protected by what is going on in the rest of Europe. Because we had situations in the United States
for example where in many areas the level of vaccination has been very very low and yet no polio occurs because the chain of transmission all around so that that kind of reasoning did not attain. But that this was an absolutely fundamental point that had to be resolved was very great and furthermore it had very practical importance for public health policy decisions. In other words when finally public health groups came around to decisions yes we should use mass vaccination with oral vaccine the question was should those who have had three, four, five doses and had Salk vaccine, should they also receive the vaccine, and the answer was yes. Even if they are protected against paralysis from the social and public health point of view their intestinal tracts are places that can serve as a place for virulent polio virus to multiply and unless their intestinal tracts are also rendered resistant by infection then you will not be able to achieve a break in the chain of transmission.

Q So it is not only the way Sabin vaccine is given by mouth that is the important element.

A Oh there are many other factors in developing a strategy and all of this had to be based on knowledge acquired, you see. Alright

Q Go ahead.

A Now, having listed these nine points for Dr. Janney I said since the series of tests carried out in April 1956 with the strains that were at that time believed to be the best. Let me digress here because what happened was that the strains that I thought were the best in April 1956 I subsequently
decided were not the best and changed. So let me start over again.

Since the series of tests carried out in April 1956 with the strains that were at that time believed to be the best that could be obtained it has proved possible to obtain still greater attenuation of polio virus. This was achieved by testing the progeny derived from many individual virus particles. This was the prack technique was introduced. Studies of the genetic homogeneity and behavior of these more desirable strains are now in progress in our laboratory. When these tests are complete and it is possible again to select the optimum strain for each of the three types I expect once more to prepare relatively large quantities of each of them and carry out another test on volunteers at the Chillicothe Reformatory. I think that you may have heard from the warden at the Chillicothe Reformatory that the manner in which I have carried out these tests has interfered very little or not at all with the work carried out by the inmates who served as volunteers. I should also like to say that the cooperation that I received from everyone concerned at the Reformatory has been most gratifying. Many thanks for your help and kindest personal regards.

Q That is an extraordinary letter.

A Well my comments in between--

Q Are even more important.

A I think this summarizes a great deal without going into the details of

Q You don't--I've gotten the details. I've gotten the details.
A So the question is in our outline what is--

Q Dr. Sabin, as I listen to you now I get the picture in my own mind of you as a kind of symphony conductor. In the one hand there are these tests going on at Chillicothe. And at the same time there is work going on in the laboratory for looking through various ways for new attenuated strains. Could you tell me something of the development of that work?

A The work that was going on in the laboratory for other attenuated viruses was not just for new but there had to be a criterion of decision. What would make a new attenuated strain that we might obtain either ourselves or by somebody else better than the one we had. So what were the basic criterion. There were really basically two criteria. One was a strain of virus which on intraspinal inoculation would have less paralytogenic activity than those that we had already selected. That was a criterion. We could test it. I used thousands of monkeys for this. Number 2 was a strain of virus which on feeding to chimpanzees first it was always our step in between. And ultimately to human beings would after extensive multiplication in the intestinal tract have less reversion so called quantitatively to particles of virus with a greater neural virulence than the one that was fed them. As I pointed out before it never went all the way. So those were two criteria. That was what we were looking for. Because I felt that before a decision is made on any one strain we must have the best that we can by these two criteria. So let me first of all concentrate on the one criterion for
which we didn't need human beings namely we could just do intraspinal neural virulence tests in monkeys. Without going into the reasons why, we used several approaches first in the laboratory which consisted of trying to select viruses that had unique properties. One, could we for example out of hundreds of millions of virus particles in monkey kidney tissue culture fluid find some that would multiply in eggs and then perhaps have totally different properties. Could we for example fine particles that on intracutaneous injection in monkeys could select out, would have the special propensity to multiply at the local area. I have already said that after intramuscular injection you needed in the human beings you needed very high doses. It was not very effective. And so we used these two approaches and Dr. Channick who was in my laboratory at that time particularly had that section because among my different associates at the time we had to divide up the labor. This brigadier general had one major job. This colonel had another major job. I think militarily again, you see, the division of labor. Well all I can say is that a very excellent job was done on attempting to select variants in chick embryos and also by intracutaneous injections, serial passages in the skin of the monkey. It didn't yield what we desired. The details of quantitative studies are on record although I never had a chance to publish them. I mean if I were the kind of person who stopped thinking about the challenges of today and tomorrow, I would stop now and I would sit down and write a monograph for history in which I
would include in detail all the tests that were carried out that were never published to show exactly how extensively we went into the search, you see because I only had time to write up summaries. Alright, so I think even here now I will say that it was a blind alley. It was done. It was a blind alley.

The second question that could be approached first of all without using human beings namely a virus, a strain of virus that might be called more genetically stable, you know genetic stability is a term that virologists use and I think any virologist who uses the term genetic stability and is a very good virologist and I know some of the best among my colleagues really is not genetically sophisticated. He may be an excellent virologist but he is not genetically even though he may be very good because in my judgment there is no such thing as genetic stability because everything that is alive and that multiplies and depending on the number of generations the number of particles that are produced during the process of multiplication the chances of running into particles of virus or of bacterium or anything that has different properties becomes greater the larger the number. Moreover the question is not just whether such particles appear. The question is how many out of a million or out of ten million or a hundred million have this capacity. And how can you select that one out of a million or ten million or a hundred million or it could even be one out of a thousand so the term genetic stability is relevant but not quite accurate because what one is looking for is the frequency with which such variants may arise. So if we use genetic
stability in that sense this is really what we are looking for in a sophisticated way. So that if one virus particle. It could even be the progeny of a single virus particle has a tendency to shoot off a certain kind of mutant. We will call it mutant x with the frequency of one in a hundred or one in a thousand and if it is an undesirable mutant it is not as good as one that may do it with a frequency of one to ten million or one to a hundred million, you see so this is a quantitative thing. The secondthing in this relative behavior population genetics not stability as I said before. We are not talking of something that is stable. Another thing we are looking for is whether the type of mutants a given virus particle gives off in its progeny has a selective advantage in the medium in which it multiplies and here first of all it is in the tissue culture fluid that we use and then we have to consider where it will be used in nature in the intestinal tract so some of it we can measure in vitro in the laboratory and some we must wait until we get to the human being. Now in order to search for a strain of virus that would become the seed virus for a vaccine to do this kind of work it becomes absolutely necessary to work with the progeny of single virus particles. When I began this work the only way we could approach and we did use this to getting the progeny of single virus particles was by doing multiple dilutions way out one to a hundred million. One to a billion inoculating very large numbers of tubes and selecting the progeny of selecting those cultures that occurred in tubes inoculated with dilution where
maybe one out of twenty only which means that in the 19 other tubes there were no virus particles to initiate multiplication enough to measure. Whereas in that one there was. But on the basis of distribution curves which I am not going into detail technology the probability under such conditions that we really have the progeny of a single virus particle is not very great. It could be two, it could be three so what do we do. What did we do because this was in our first experiments that we finished in 1953 and then published. So to increase the probability that we have the progeny of the single virus particle we take the progeny from the first very high dilution and again dilute it a hundred to a thousand times to purify to get away from some other and make another such passage. And then a third one. But even so that is not the optimum way to get progeny of single virus particles as had already been found with bacteria farge where plaques had been known for a long time and even with plaques you are not always sure that when you have one plaque produced by a farge particle or one plaque is then came in with virus particles is really the progeny of the single but the probability is very high. And when you do it what's called a strictly plaque progeny purification you increase that very much and particularly if you don't study just one. But if you study the progeny of many plaques, single plaques you increase the probability. While I was doing this work Dubeckow and others began to apply the farge techniques to virus particles. Actually Dubeckow if my memory serves me correctly first did it with Western
equine encephalitis to work out the technology. And then the technique for producing plaques he and others then were done by others. I mean we couldn't do everything and this is an example of how concurrent work in other fields leads to when utilized in a directed objective problem oriented research you feed in knowledge that comes from the periphery. So when the plaque procedure, plaquing procedure for polio viruses had become well developed I decided that in my search for a virus that would have the most desirable genetic properties on the basis of the two criteria that I mentioned I would have to begin to study the various strains that I would study by plaquing them and obtaining the progeny of individual plaques and also then to test them intraspinally in monkeys. To test them intraspinally to test them by feeding and so on. Originally I began some collaborative work with Dubeckow on this but that did not lead very far. I had to then set up the work myself in my laboratory. And then there were all sorts of factors that we had to learn incidentally on what favors the appearance of plaques because the plaquing procedure itself is a selective medium. That if the concentration of let's say the pH of the overlying medium because how do you do a plaque. I must I think--

Q Oh please.

A You have a on a plate or in a bottle you have a single layer of cells. Let's say monkey kidney cells which is grown and as a uniform sheet nice and beautiful and you watch it very carefully and you dilute your virus culture in a different dilution all the way out to a point where perhaps you would have
only a few plaques. Each one far enough apart. What happens
what is a plaque. At this point I am not speaking to colleagues
but to other scientists who don't know what the hell a plaque
is. When a virus particle capable of producing an infection
falls on a cell that is susceptible it begins to multiply in
that cell but if you don't cover that cell sheet with something
that would prevent the virus from just spreading all over once
the first burst. Let's say the first cell gives rise to a
hundred or a thousand infective particles when that one
particle multiplies. Unless you prevent it from infecting
all the other cells on that on that cell sheet you never know
which is the progeny of the one or which is the progeny of the
subsequent one. So what was done at that time is that you
cover it with an agar overlayer. And that prevents the spread
of the virus to many other cells in that cell sheet. But it
does not prevent the spread of virus by contiguity
you see so that where the first cell that was infected yields
a certain number of virus particles the adjacent cells are
infected but all the other cells in the bottle are not. So
you get a little clear area where the cells have been destroyed
and if you want the progeny of a single plaque, of a single
virus particle you then go into the bottle or the plate with
a fine capillary and you put it right over the agar and you
suck it up and then you have the virus that was produced in
that small area. So you have to dilute the virus in such a
way that you don't get a thousand plaques. You don't even--
you have to dilute it in such a way that some bottles have
only one plaque. Or that the distance between two plaques is far enough that the virus couldn't diffuse. Ideally you choose a bottle that has only one plaque on it. Alright, or the distance must be very good. Now this is a very important part of the technology. But then it turned out as studies showed that I had to discover because you play with it that the concentration of sodium bicarbonate in the medium which was incorporated in the agar overlay itself was a selective factor. That when the acidity of alkalinity under certain conditions that very virulent virus altered strains of different virulence neural virulence now it made no difference what the pH was. But if it was too acidy then these more highly attenuated strains would not form plaques. I mean there were all sorts of things. Well at any rate I mean having worked through the forest of variations and additional factors which were found to be associated with neural virulence that then served as a basis for monitoring cultures we obtained and produced the progeny of triply purified plaque virus of the strains of virus we were studying and a certain other candidate strains. And that a tremendous amount of work because after triple plaque purification we then would grow up a certain amount of virus enough for the various kinds of tests that you would do neural virulence in monkeys and subsequently let's say potentially for feeding. But from each strain that we already had that had been selected by the terminal dilution technique purification before it was necessary to have the plaque progeny from a whole lot of different plaques, from at least ten. Not just
take one and say this. Because how could you compare genetic variations in your culture unless you tested the progeny of a number of different plaques. So this you see multiplied the work extensively. And then of course we began to learn when we did that that not all plaques had the same size. Now what is the size of a plaque mean? Supposing on the third or fourth day after you do such a test you find that one plaque measure one millimeter in size and quite a number of cells involved in the one millimeter and another plaque measure five millimeter. What does that mean? It means that the virus in the small plaque has a lesser capacity to spread from cell to cell and probably produced less progeny during multiplication than the other one. At least it was a difference because for viral genetics the size of the plaque already became a factor, a genetic marker and so we had to face also the question not only of studying the progeny of individual plaques triply purified but studying the progeny of small plaques and large plaques. This already had become a factor in viral genetics which you probably discussed in this conference.

Q Go ahead. I don't want to interrupt.

A No I must. Let me develop this.

Q Please develop it. I have a series of questions.

A Now ultimately with all of these considerations we had a very large number of triply plaque purified progeny from strains of virus that we had previously studied extensively in human beings and some others. And we tested them intraspinally in monkeys by a technique and the technique of intraspinal testing itself is important and there was a lot of controversy
and I wrote a paper on that but never mind. I used the same technique. I always inoculated the monkeys. And it does not depend on faith or something on me. It is something that you can test because when you sacrifice the monkey after three weeks or four weeks and you make many many sections from the spinal cord you can see exactly where you put in the virus. It is not a question of taking on trust. Did you put it in through the anterior horn. Did you blow the grey matter out by your inoculation by poor inoculation did you put it in in the white matter in which case you have to discard that monkey. In other words there were very fine technical guidelines for significance. And I did all the inoculations. Hundreds and hundreds of monkeys inoculated intraspinally with different plaque purified and I found and this was a big departure that when I had used strains of virus that had already been triply purified by terminal dilution in which I had used in human volunteers that when I took the progeny of individual plaques that I had a spectrum of activity by the spinal route, that they were not all the same. So then I had to select a virus that I thought on the basis of these tests and there were at least ten monkeys inoculated each which had the best or the least rather paralytogenic activity on inoculation directly into the anterior horn of the gray matter of the spinal cord. That is why the strains that were ultimately selected have certain letters on it. For example the name of the strain and then 2-ab. What does that mean. It means plaque number 2 you see it originally started and the designation of the subplaques because when I plaque from the first one there
were different kinds of plaques and the one designated "a" were used and then there were "b". So these designations were the plaque numbers that won out.

END OF TAPE