A Spain there were no political considerations involved there except that Spain was having a great deal of polio which was increasing in the late '50s more and more and finally they decided to have a mass vaccination program with vaccine I think they were getting from England at the time, again in December. And there was the most dramatic immediate effect of the mass vaccination program comparable to that which was found in many many other countries. But, from the point of view of political interference and political problems in decision making France represented an interesting example.

In France an esteemed colleague of many years, Pierre Lapienne in charge of the virus section of the Pasteur Institute was making vaccine, killed virus vaccine that was no different from the Salk vaccine but he called it the Pienne vaccine. So in France the Pienne vaccine was being used. The Pasteur Institute had a special subsidy. It was the only one making it. Getting quite high prices for it. And as a matter of fact was able to support the research program of the Pasteur Institute to a large extent from income. That wasn't the only vaccine they were making, of the Pienne vaccine. And when world pressure and world acceptance began to build up for the use of this vaccine, the, a campaign was organized because it must have been organized because there were leaflets that were going around, calling the live polio virus vaccine at that stage. This was '61, a vaccine that only communist countries would use. It was a communist
vaccine. This is not for decent countries and highly developed countries like France and others. Of course it wasn't yet being used on a wide scale in the United States although hundreds of thousands had already received it to fight epidemics. You know. Not hundreds of thousands. There was one epidemic that had to be fought in '61. So this is '61. This is early. It is of interest that before that, early in 1960, when it was accepted in the Soviet Union and there were reports from millions, hundreds of millions being used that I had two requests for the strains, one from Pienne, Pasteur Institute and the other one from a regular biological pharmaceutical house Maurier of Leon. And Maurier went quickly to work and by the middle of '61 or whenever it was they already had vaccine ready for use. But couldn't get approval. There was a meeting that was called together at the Maurier laboratory in Leon in which LaPienne was also present and in which he said that he had asked me for the strains and that I did not give it to him.

This was absolutely outrageous because I had the correspondence to prove that I had sent him the strains. He merely did it for the record to say that I have asked. And he received it. But he had done nothing about it. What happened then was that the minister of health for France called in Professor Treffwell who was the director of the Pasteur Institute and Dr. Maurier the president of the Institute Maurier, a pharmaceutical house, and the story is as it was told to me by Maurier, here is what happened in the minister's private office. This is 1961 was somewhat like this. Professor
Treffwell was saying to the Minister in a sense Mr. Minister, if we can no longer sell the La Pienne vaccine we will have to shut down the Pasteur Institute. We depend for our continuing existence on the income we have from the La Pienne vaccine. He didn't say anything that all he would have to do would be to—if you were going to get it for free. But you could hardly charge the prices that they charged the La Pienne vaccine, a dollar a dose or something like that compared to pennies.

And Maurier was saying here is a vaccine. Used very extensively we are ready. We have it. It is tested. Mr. Minister will you give permission. And Treffwell. And finally Maurier told me that he turned to the Minister and said to him in French, Monsieur La Minister, dit moi qui est le commerçion maintenant quoir ou l'Institute Pasteur. To translate for the record, Dr. Maurier turned to the minister and said Mr. Minister tell me who is the commercial man. Who is just a businessman now. Myself who is a business man or the Institute Pasteur who is arguing and this I am adding on. Who is arguing for the continued use of what is called the la Pienne vaccine simply because they need the money to survive. Well as a matter of fact it was not resolved for a long time in France. And ultimately with a new director the job of making all polio vaccine from my strains was begun again with new material. And now the Institute Pasteur as well as the Institute Maurier are in production accepted production in France with all of these political considerations that played a part.

Q I will stop there. Dr. Sabin, at this point I would like to switch gears and try to address a general question.
That is it is apparent now that when one looks over the record the first thing that strikes one is the multiplicity of knowledge that one has to acquire for solving the problem of producing a vaccine and I don't know whether I am using a right word or not but there were any numbers. I hate to use the word ancillary because I feel it was right in the center of your studies but an extraordinary amount of study that you did on various, of the enteral viruses, of which we didn't know very much about at that time. And I wonder if you would address yourself both generally and specifically to this question of the multiplicity of knowledge that one has to acquire.

A I wonder if I may interpret this question as applying not only to enteral viruses but to use an example of other essentially fundamental problems.

Q Fine.

A Requiring understanding. One of the problems that came along and the studies of the various factors that differentiated attenuated polio viruses from paralyzing polio viruses fully realizing we were dealing with a spectrum was to try to understand what corollary properties they had that might let's say parallel or perhaps even be linked to the fact of their attenuation. Now this can be asked as a question in search of understanding. Differences between attenuated polio viruses but I also visualize that this was the necessity and you might say this is a general principle that when you ultimately came to the end point where you had to begin to make such vaccine on a large scale you would need to have every
possible marker. This is the marker used in the genetic sense. That would differentiate the behavior of paralyzing strains from attenuated strains that could be tested without having to resort to tests to human beings all the time. One of those markers that I was led to investigate actually originated from a casual observation by Francis and Chew that using the mouse adapted Lansing strain of polio virus they could show that the grey matter. I don't remember exactly whether they scraped away the white matter. But the gray matter of the spinal cord let's say of mice had a special affinity for the Lansing virus. It was not a neutralizing capacity but it would take virus out from the let's say suspension containing a certain concentration of virus in a way that let's say the white matter. I don't recall their work now in great detail but the way that would be done by such virus so that here was a suggestion that perhaps in the tissues and the cells that were susceptible to a virus by comparison with those that were not, there would be certain kind of specific receptors because this is an established concept now. But there would be certain kinds of specific receptors in susceptible cells that were not present in non-susceptible cells and the question was also whether or not there was a real specificity and whether the receptor content or the nature of receptors could be shown to be different for a very virulent polio virus and the highly attenuated progeny selected out from the mutants arising from it. And I undertook to study this question and I would like to recapitulate here the basic findings as they were contained in my reports.
In my annual report to the N.F.I.P. for the period of July 1, 1955 to June 30, 1956 I summarized our studies on this question under the following heading. Combining capacity of nerve tissue suspensions of different species with virulent and attenuated strains of polio virus. This study was undertaken to determine whether or not by using type 1 tissue culture virus and the more precise quantitation permitted by plaque counts, it would be possible to confirm the observations reported by Francis and Chew with the type 2 Lansing polio virus in mice indicating that the grey matter in the nervous tissue of species that are susceptible to poliomyelitis contains a substance which specifically absorbs and combines with polio virus. If such confirmation could be obtained it seemed important to determine whether the grey matter of chimpanzees would still combine with or absorb those attenuated strains of polio virus which had been proved by repeated tests to produce neither paralysis nor lesions after inoculation of as much as 5 million tissue culture infective doses directly into the spinal cord.

An aside from the report now. The basic question to recapitulate this is whether the virus was so attenuated that it lost its capacity to combine with the receptors in the grey matter of the chimpanzee. This would be a terrible important thing. I go on with the report to say.

The essential results that were obtained are briefly presented in summary under (b)(4). Hold a moment. Summary just mentioned. ...Is on page 8 of the report for the same period and it reads as follows.
Studies on the combining absorptive capacity or you might say combining capacity of suspensions of grey matter derived from the nervous system of primates. That is monkeys, chimpanzees and man, and from the nervous system of dogs and dogs are not susceptible to polio. Using type 1 polio virus and the plaque technique confirm the observations of Francis and Chew with the type 2 Lansing polio virus in mice that the grey matter of primates susceptible to poliomyelitis contains a substance which combines with polio virus in vitro. While the grey matter of non susceptible species such as the dog does not contain such a substance.

As an aside I would say here in modern parlance we call these things receptors, cell receptors and current biomedical research and physiology and everything else, cell receptors play a most important role in almost every life process. Now I continue with the report. However our studies also indicated that means in addition to confirming the observations of Francis and Chew, also indicated that the grey matter from several parts of the cerebral cortex of the monkey which has never been found to exhibit polio lesions, or yield polio virus in monkeys that have been paralysed and almost dead. Also possessed a similar capacity for combining with polio virus. Now my comment on this is not in the report. This immediately indicated that nerve cells, the cells in the grey matter of susceptible species could have a combining capacity with a virulent polio virus and yet lack something else which is necessary for multiplication of the virus and for the destruction of the cell by that virus. So this expansion of the work already
indicated a new phenomenon namely that there was a double difference in specificity of action of polio viruses. One, the presence of receptors. No receptors present in grey spinal cord matter of non susceptible species, spinal cord let's say and presence in susceptible species, like monkey, chimpanzee and man. But this second finding shows that receptors alone are not enough because areas of the cerebral cortex not affected by polio virus, no virus, no lesions also had these receptors so this is an immediate important orienting finding.

Furthermore. I am back to the report now. Furthermore, suspensions of grey matter from chimpanzee spinal cord did combine with the attenuated type 1 LSC virus which had no demonstrable activity on spinal inoculation of the largest doses in chimpanzees as well as with a fully virulent type 1 virus which has been shown to be paralytogenic on spinal inoculation in chimpanzees. Now again there is an aside. So in response to the original question that was posed, was it possible that the reason these attenuated strains had no effect on chimpanzee spinal cord was because it had--the virus now had lost the combining receptors for those in the cells the answer was no. In other words they didn't produce any lesions at all in the spinal cord of chimpanzees not because there were no receptors but because there was something else involved.

However, in a number of tests the suspensions of grey matter derived from monkeys, chimpanzees and human beings there was a suggestion that the same, and I want to emphasize that I used the word suggestion. Not evidence. There was a suggestion
that the same quantity of tissue combined with more avirulent virus than of attenuated. Now if that is really so there could be a quantitative factor of the number of receptors that the virus had for a given cell. And perhaps that could make a difference. In one simultaneous experiment with a suspension of grey matter derived from adult human spinal cord there was definite absorption of the virulent virus but no absorption at all of the attenuated virus in one. However, this could not be confirmed with preparations from the spinal cord of children which may have had much less white matter admixed in the suspension. Because in order to get grey matter. How do you get grey matter out of a little spinal cord. This is an aside from the report. You freeze the spinal cord on a block of dry ice and you shave like a sculptor you see you shave the white. Now you hold it with a forceps. You scrape away the white matter until you see only the grey. Now, you use the scrapings as your control because the white matter is not supposed to have any in it. It doesn't actually. But in doing such an experiment with human tissue, human spinal cord it is conceivable that you can sometimes leave more white matter on and therefore there would be less combining capacity. At any rate the important point was that as I say it could not be confirmed with preparations from the spinal cord of children which may have had much less white matter admixed with the suspension. The original experiment was done in the spinal cord of an adult. Of course we obtained this material from routine autopsies on conditions other than polio. But I dare say that there is another possible hooker here that may be
there was some antibody because we didn't know whether they were persons without antibody for type 1 or with antibody for type 1. The study carried out thus far indicated that quantitative aspects are of the utmost importance. For example, it was found that extracts of grey matter which did not absorb the virus that means from non susceptible species you see had no specific absorption which did not absorb the virus seemed to yield a substance which increases the number of plaques in the same suspension as compared with that obtained in simultaneous assay (?) in the absence of such extracts. The efficiency of detecting polio virus by plaque technique was apparently effected by some substance in the grey matter. We subsequently found for example that things like pH and other things had an effect. So here is the importance of the quantitative aspects.

It was decided therefore because grey matter from mouse susceptible animals could increase the cell count. The plaque count. The number of infectious units present. It was decided therefore that further studies along this line should be postponed until certain aspects of the technique are worked out. In the meantime however it is evident that the complete absence of paralytogenic activity in an attenuated strain for chimpanzees is not correlated with inability of chimpanzee grey matter to combine or absorb such an attenuated virus. Now when I get back to the other summary again I say the chief conclusion which can be drawn thus far is that complete lack of paralyto-

-of genic as well as pathogenic activity an attenuated strain
in the nervous system. I repeat here. Is not correlated with the failure of this virus to be absorbed with or combined.

Q You know--

A Let me just see one moment more. You want to shut it off a moment.

Q Yes.

A Now there is something else that I said. Unless quantitative aspects which are still to be worked out are of crucial importance. By quantitative aspects here I mean the number of receptor-combining groups on the attenuated virus as compared with the virulent virus. It would appear that events which occur subsequent to the combination of the virus with the specific substances in the nerve cells are more important in determining the failure of a given attenuated virus to multiply in or damage the nerve cells. This tentative conclusion would also appear to be borne out by the observation that suspensions of grey matter derived from the occipital (?) temporal and frontal portions of the cortex of monkeys and of chimpanzees in which the most highly virulent polio viruses characteristically produce no lesions and no evidence of multiplication nevertheless exhibited the capacity for specific absorption of polio virus. Now this is a bit of repetition here but it brings out and this study was published incidentally, subsequently that it was necessary to study this and interestingly enough the study of receptors on susceptible cells subsequently became a model for other studies, studies with enteral, other enteral viruses, coxsackey viruses, echo viruses and it has a basic importance in understanding the
interaction between viruses and susceptible, and non susceptible host cells. So this was one aspect of the problem. Only one.

Q There are a number of things that sort of strike. At least they strike me and that is the refinement of techniques for detecting polio virus. You mentioned the necessity of knowing the pH, the scitopathogenic effects in tissue cultures and sometimes you wouldn't get a scitopathic effect and you would use pH technique and you would get--

A Well of course this again it turned out that using the combining capacity of attenuated polio viruses with the nerve tissue was not a practical. Or at least it wasn't worked out to a point of being a practical marker for differences. The difference in pH acidity and alkalinity of the overlay over the tissue culture in which plaque counts were made became an important part of it because it turned out that at a certain alkalinity you would get the same number of plaque counts for the attenuated viruses and the virulent viruses but at a reduced alkalinity the attenuated viruses would or the acidity, a certain acidity, the attenuated viruses wouldn't whereas the others did just the same. Now please hold that a moment. I want to refer to--.

The question of differences in the number of receptors between attenuated polio virus and virulent polio virus particularly on chimpanzees and cynamologous monkeys was pursued further. Because I said before that using a standard amount of grey matter, half a gram, there seemed to be a difference in the combining power of the grey matter with virulent virus and attenuated virus, less of the attenuated
virus being combined with the same material, aliquot of the same grey matter. And I pursued this study further because a publication in a special symposium that was held by the New York Academy of Sciences in 1957 and is published as a special publication under the name of Cellular Biology in Nucleic Acids in Viruses. On page 121 in summarizing different activities of attenuated polio viruses I described this study with grey matter. And I found the following additional results that were obtained.

To start off with I said it is evident that a quantitative difference exists between the combining capacities of the highly virulent and high attenuated strains. A difference that becomes especially apparent when the quantity of grey matter was reduced from half a gram to two tenths of a gram. Thus while two tenths of a gram of grey matter from the spinal cord of the chimpanzee combined with 95% of the virulent virus particles it did not contain enough of the specific substance to combine with any of the highly attenuated virus.

I would like to comment as an aside here on this observation. The question is where are the receptors changed. The virus or the cell. Now it is obvious that two tenths of a gram of grey matter of the spinal cord of the chimpanzee has enough receptors in it for virulent polio virus to take out 95% of the virus particles. But there is not enough there to do anything to the attenuated. It would suggest therefore that either the attenuated virus lacks certain kinds of receptors that there are multiple cell receptors that a virulent virus has not just—we are not dealing with a single receptor but say we are dealing with x number of receptors for certain cells.
The virulent virus has the more. The attenuated virus has some but not others so that when you reduce the concentration of cells the virulent virus is the one that is lacking certain receptors. Because the chimpanzee material is the same. We've got a constant here. The receptors are the same. The virulent virus combines therefore the change must be in the virus. And that would suggest very strongly that quantitatively there are probably multiple receptors for combination with the virus particle and that in the process of attenuation it loses some of those receptors. An alternative possibility is that the total number of receptors. It is not that they are different but that the total number of receptors for a susceptible cell on the virus particle now differs. It is the same kind of receptor. But the total number on the virus particle is less. So that the virulent one has a great many of these receptors, to combine with things sticking out the cell membrane whereas the attenuated one has lost not all of them because if you use five tenths of a gram instead of two tenths of a gram you can still demonstrate combination although not as much as with the virulent. And if I would have to choose now on the basis of this limited information as to whether or not we are dealing with different kinds of receptors, qualitatively or merely quantitatively I would be more inclined to think of a quantitative difference in the number of receptors that an attenuated strain has as compared with a virulent one. Now since there is a difference of the same virus in its behavior in the spinal cord of the chimpanzee and of the spinal cord of the cynomologous monkeys the next experiment was done with the spinal cord of cynomologous
monkeys because if the concept of a difference in multiplicity of receptors, you could have also a difference in the number of receptors in a chimpanzee spinal cord, in a cynamologous spinal cord, in a human spinal cord and in the spinal cord of a dog. A dog would have none. A cynamologous monkey may have the most. A chimpanzee less. And a human still less. And this would fit with the emerged finding that there is a difference in susceptibility to the same virus, attenuated virus. The virulent is the same across the board.

So let's see now. Let me read the results of the tests with cynamologous spinal. A comparable result was obtained with grey matter of cynamologous monkeys. In one test performed with five tenths of a gram of grey matter from an adult human spinal cord approximately 75% of the virulent virus but none of the highly attenuated virus was found. Now this then shows that we are dealing not only with a question of whether the virus binds to a cell or not. It is not an all or none process. And then as I said before, other events determine. Probably there are other events also. But it is now clear from these more quantitative studies that there is indeed a difference between the number of receptors for virulent and for attenuated viruses that are possessed by human, chimpanzee and cynamologous spinal cords.

Now I continue. When grey matter from new born children or adult basal ganglia was used in other tests, combination with a highly attenuated virus could be demonstrated also. Since the highly attenuated viruses produce focal polio lesions in the site of intraspinal inoculation in a certain proportion of the monkeys we can assume that limited multiplication of the virus occurs.
But this increase is insufficient to permit it to spread to a sufficient number of other neurons to produce paralysis. This limited capacity to spread is borne by inability to demonstrate any virus in cervical portion for the spinal cord, etc.

The main point here is that those studies that indicated a certainly a difference in what would appear to be a quantitative difference first between human and cynamologous and chimpanzee and it may even be in human beings of different ages you see. And that there was a difference in receptors. Actually it did not get to be. It did not become because a tool for monitoring the, this particular property in production lots of vaccine. Because a much better tool was found. The better tool was found out of an observation in the course of growing polio virus in bottles, in tissue culture bottles. On certain number of culture fluids of attenuated polio virus having a very low yield. In certain cultures grown in certain vessels. Let's say that the expected yield would be around 50 million infectious plaque forming units per cubic centimeter on other occasions it could turn out to be 20 to 30 times less. And that was a very disturbing difference. Well, in observing some of these differences, one of the factors was this. That in certain vessels the escape of the CO₂ from the fluid because there is more air over it makes the thing more acid. And under conditions where you have either a higher concentration of sodium bicarbonate of the same concentration where the pH does not become very acid it could be one of the differences. And a systematic study was carried out to determine then whether there was a difference. Whether this applied to all polio virus, virulent and attenuated as well or only to attenuated.
Could this be another genetic marker. And it turned out to be a genetic marker. Because carefully controlled studies using different concentration of the sodium bicarbonate in the culture fluid and following the pH and determining the concentration of virus produced at certain times after infection showed that with the virulent polio virus, this for example, I have tabulated in this article. I mentioned a study with the Leon type 3 virus where I have the parent and I have the ultimate selective progeny from it. It was a very clean cut difference. The virulent virus didn't make any difference what the pH or the concentration of sodium bicarbonate was. On the other hand with the progeny derived from it it was evident that using a low concentration of sodium bicarbonate in a medium that would become quite acid that the yield of virus could be--of the attenuated virus 20 to 30 times less. Now that is not only an interesting genetic marker but it becomes of great practical importance because it means that when you produce vaccine for mass production, in mass production that instead of having in the same volume one million doses, you have 30 million doses. That is a lot of difference. That is 29 million doses, just by observing the pH. And so this information then became practically applied in the overall complex process of getting to the end point of use and production. And the following way.

1. You could use the number of plaques that appeared under alkaline and acid medium as a monitor and secondly. By carefully controlling the pH of the medium in which the virus was being made for the vaccine you would have a higher yield.
Q So the quantitative studies are really a necessary part of your research.

A Not only the quantitative studies. I think what I am stressing here and perhaps what you are trying to stress also is that in arriving at an end point that merely involves ultimately in a practical way of putting a drop of fluid on a piece of sugar or in the mouth of a baby, there are an awful lot of fundamental steps that have to go through to provide you the basis of knowledge to make it practical. And the importance of this is that there is very loose talk about what is basic research and what is applied research. And as to the relative importance of each. My point is that it is used in a very obscuring way, those terms. Certainly all of this infrastructure of knowledge necessary to achieve a specific objective, yes or no, reach an end point of decision, of having a vaccine that you could use involves a tremendous amount of what is fundamental research giving us an understanding of differences between virulent and attenuated polio virus. That is fundamental research. But some of these questions would never be asked in a thousand years perhaps if there weren't an end point, a specific objective.

Q Well you know, sometimes the questions don't come out of your own work but really observations that you make in other people's laboratories.

A Oh this is a center point now. There is nothing. There isn't a single piece of work. And I will leave out Genesis as--there isn't a single piece of work of one scientist that wasn't built on the shoulders of another scientist.
Q Well I was thinking of a particular observation and a particular piece of work that you did and for example, in I guess it was. Let me check the date. It was sometime in 1956 you visited Wilson-Smith's laboratory in London and you learned that he had a stable line of embryonic rabbit kidney cells which was found to be susceptible to all three types of polio virus and you began to work with these cells. Can you tell me (a) why you started to work with these cells and what you hoped to accomplished and what the ultimate outcome was of this work.

A Well, practical considerations were of course very important. Nobody in the first place has ever been able to grow polio virus in rabbit cells. So if these were rabbit cells they had to be a mutant of some sort. They were very unique receptors for polio virus that the others didn't have. So I was interested for that reason. The more practical reason was this. That if these cells would really be an acceptable source of virus for mass production of vaccine, my God, just think how wonderful it would be not to have to use monkey kidney cells if they have a stable line they can carry in.

Well I undertook those studies first of all because I didn't question the possibility that they might not be rabbit cells. After all, I had the greatest regard for Wilson-Smith. But this merely shows that just because Wilson-Smith was a great man, things that happened in his laboratory didn't necessarily reflect on him. But at any rate, I carried out studies with attenuated polio virus in the so called rabbit kidney line and it did in fact multiply beautifully, extensively.
So then I proceeded to do tests for neural virulence of serial passages of the virus in these embryo rabbit kidney cells. And I found that it was more neural virulent than virus similarly passaged in monkey kidney cells. So the practical part immediately dropped out. But other people also for reasons of general interest began to study this and of course other work began to be reported of contaminations occurring in laboratories of all sorts of tissue cultures that were not mutants but merely that the helo cells if helo cells were somewhere around, one cell could somehow or other contaminate the culture then you would get that out and you would think you've got a mutant. As a matter of fact one of the things I found that contributed to this was a horrible habit of many technicians and some Ph.D.s in tissue culture laboratories. Pasteur pipettes, cotton plugged Pasteur pipettes I used very often to suck up something and transfer it from one tube to another and there are—you use a rubber bulb which you attach to the Pasteur pipette to suck up the material. And I found in quite a number of laboratories that people walked around with a rubber bulb in their lab coat. They would very carefully change sterile Pasteur pipettes but they wouldn't change the bulb. And so it was possible very often to contaminate the bulb. Because after all the cotton plug can serve just so much. And then you put on let's say a bulb that has been contaminated with a helo cell and you transfer rabbit cells and one of these helo cell gets in there and it grows on the wall. And this is what happened because ultimately extensive studies in which I was also involved proved that this embryonic rabbit kidney line was in fact a
human line of cells. And subsequently by more refined tests, that it was a helo cell. So some of these things, what did I learn from it except that somebody--

Q Be careful.

A Somebody did that. No. No. More. That somebody used bad technique in Wilson-Smith's laboratory as well as in many others. It happened in many places. I learned the following. That the maintenance of the neural virulence in culture outside the body is influenced by the type of cell that you use for propagating the virus. And that is why I was always adament that the cells in which the virus has been standardized and which it has been multiplication has been used for all this. That they must be used for production. And when people first came along with human deploid cells I was opposed to its use because I said my previous experience with a human cell. Alright. It was a malignant cell, helo cell, show there can be changes in neural virulence. And I was also opposed for reasons that we didn't know enough about possible viruses that might be carried in human cells that would have an affinity for human. But later it was indeed found that while progress in molecular biology and detection of perhaps residual viral umpogenic genones (?) was not a problem in these deploid human deploid cells, normal cells but rather it turned out to be evident that unless you use monkey seed virus for just one passage in the human cells you got into trouble with neural virulence. And you even got into trouble with neural virulence in using human cells for
propagation of the vaccine virus if you inoculum was too small so that you would have to have a number of generations of the same culture to destroy all the cells. So in a sense the information that was obtained in what turned out to be an unfortunate situation, a mistake in designation of the cell line, nevertheless served to point up the importance of the requirements for maintaining stability for cells, of the virus on cultivation in vitro at least.

Q I just want to see where we are. We still have some tape. Now one of the things that I find in this search for multiplicity. Well, that is the multiplicity of knowledge that is required was really the extraordinary and extensive work that you did with various kinds of enteric viruses. What may be coxsackies or echoes, and I really found that your laboratory at the same time you were working on attenuated polio viruses half of your work is taken up with these echo viruses.

A Well the whole problem of echo viruses and coxsacki viruses was a direct corollary of using the tissue culture technique for isolation of polio virus and in field studies on human material in our own laboratory first in the search of naturally occurring attenuated polio viruses there turned up a great many viruses that produced the scitopathic effect often the same as polio which didn't turn out to be polio. In other laboratories that were studying material from patients or from healthy children in the field, again, many viruses turned up in tissue culture that were not there. That were not polio.
So they came to be called orphan viruses. Then an effort was made to make some sense because there accumulated hundreds and hundreds of orphans and one didn't know whether they played a role in let's say in human disease or not. That is one of the reasons they were called orphan viruses. And secondly, it was also important to know how many there were, and what different kinds and what their biological character was in relation to polio viruses and whether or not they might produce disease like polio viruses or other diseases. In other words it was important to the total natural history to the understanding of the total natural history of poliomyelitis virus in nature and you might say the natural history of poliomyelitis virus in the intestinal tract. So, it was not that it was an ancillary activity. It had to be done. It is another example of understanding that had to be acquired in order to know the whole natural history of polio viruses in nature in order to be able to control them. This resulted in a great many studies. In our own laboratory we began to make antisera in the usual way of trying to find out how many different types there were. Similar studies went on in Melnick's laboratory and other laboratories and then finally a collaborative enterprise was set up through the National Foundation and it turned out the number kept growing that there was something like—I think now there are over thirty different serologic types of echo viruses in our laboratory I think we identified eleven or thirteen different types. Now this took a lot of work. Ultimately of course it was shown that biologically these echo viruses and the coccsacki viruses which originally were isolated only in
suckling mice but subsequently also came out in tissue culture. Many of the coxsackie "B" viruses and even some of the coxsackie "A" viruses were scitopathic. It turned out that these coxsackie viruses and echo viruses formed a biological group of related viruses with the three types of polio so that there was set up then one group of viruses, enteroviruses which contained a polio viruses at one end of the spectrum, coxsackie viruses and echo viruses. Now, the significance of it in nature and particularly in the relation to prevention of poliomyelitis by a live virus vaccine became very very important. First, studies in our own laboratory and other laboratories showed that many of these viruses in effect produce not only certain types of infections in human beings but sometimes big epidemics. And furthermore, that when one studied so called non paralytic poliomyelitis during the summer months which in the old days were included with in reporting with the paralytic cases giving us such figures as 50,000 cases in 1948 or whatever it was. We had 50,000 cases of polio reported from the United States, half of which turned out to be non paralytic and even more as another number transitory paralysis. It turned out that most of the non—not all but most of the non paralytic polio was not caused by polio viruses but caused by different echo and coxsackie viruses. They were even isolated from the cerebral spinal fluid which polio virus practically never was. And then let me stick to the polio part quite aside from other syndromes. So it became evident that for practical purposes you cannot call something non paralytic polio unless you prove it. And secondly with most non paralytic polio it was not
caused by polio virus. Then the other thing that arose that was less quantitatively important role is the fact that some of these viruses could also cause paralysis like we mentioned before, the coccacki A7, coccacki echo 4, echo 6. There were a number of these that were found occasionally to cause paralysis. As a rule transitory but as I indicated in my own experience in Cincinnati, sometimes not. For that reason subsequent epidemiologic classifications left out pretty much the term non paralytic polio and divided up analysis of situations of paralysis that would persist for more than a month, or two months because very often paralysis caused by echo viruses, coccacki viruses, didn't last very long. But then there was still another problem. Because it turned out in studies on children, the first studies on children in institutions and also studies in Mexico which we carried out with Dr. Romulus Alvarez continued that the intestinal tract is almost always inhabited by tremendous numbers of other viruses, echo viruses, coccacki viruses and adenal viruses and the question was whether they might interfere during a period of their own multiplication with the implantation of a polio virus. And in effect there was such interference. And ultimately we had to develop a strategy to find out how to overcome that in let's say tropical and sub tropical countries in population with relatively crowded, living under crowded and unhygienic conditions. That led to a field trial in Taluka Mexico which I will describe later. So that these studies had to be carried on. Now you might say now why should they be done in my laboratory. Why didn't I leave this question to somebody else.
Q For example why didn't you leave it to Dahldorf who after all, discovered it.

A If I would leave things to Dahldorf and if others would leave Dahldorf we never would have known anything because Dahldorf in anything about echo viruses, Dahldorf had a fixed idea that anything that was paralytogenic in mice was a coxsacki virus. I mean if you want an illustration of this I had a personal important illustration. In the first place Dahldorf wasn't very active in the field at that time. Many other people had accumulated these, were interested in finding out what do we have. But in the process of identifying them, we had one of the ones that were identified in my laboratory was echo 10. Echo type 10. I think Melnick had serologically established identifying sera for the first six. And then when we came along and those that we couldn't identify with those sera we began to make new sera. And we had seven echo, Echo type 7, echo--

END OF TAPE.