Dr. Benison: How did you come to choose the Lister Institute and what is it you expected of the institute before you came there?

Dr. Sabin: I'll have to reach way deep to my store of memory. When I finished or was coming to the end of my internship at Bellevue Hospital and I had already reached the decision that my career would be in medical research, I wanted to go for training somewhere else...abroad. It was the fashion—as a matter of fact—to spend a year abroad to get the feeling of sense of activities. You need contacts for that so I applied for a National Research Council Fellowship and I had to indicate where I wanted to work, and one of them was the Lister Institute and the other was the Pasteur Institute. The Pasteur Institute because I had been reading the papers of Leviditi and I thought I wanted to get the feel of what he was going. Well, the National Research Council which awarded me my fellowship thought it would not be wise to divide up one year in two places and so they blackballed or disapproved my going to the Pasteur Institute and the reason the Lister Institute was selected, I suppose, might have had a number of factors in it. One is the great history of having the name of Lister attached to it; secondly, the director of the Lister Institute at that time (he later became Sir John Leddingham) was a good friend of Dr. William H. Park, my patron saint, suggested that I go there and make arrangements. And, another reason was that just before there had come out reports from the Lister Institute by a Dr. Eagles that he was able to propagate vaccinia virus in a self free media. Now, during the period of my internship, I had visited the Rockefeller Institute, the Rockefeller Hospital quite often,
partly for pneumococcus reasons, partly for discussions with Dr. Rivers about my b-virus studies which I got in sideways while interning, and Rivers was very much upset by these reports from the Lister Institute because he could not fit in any concept of the nature of viruses that was held at the time with propagation self free medium. And in the typical language that he used, Rivers said to me, "Now you go to the Lister Institute and you either learn how to propagate vacinnia virus in a self free medium or else find out what's wrong. So when you come back, we can have some basis." So in a sense I was an emissary. I had not worked with vacinnia virus before. B-virus was really the first virus I had had any experience with at all. I could get some new techniques in virology. So this is really the reason I went to the Lister Institute of Preventive Medicine, not because I had made a study of where, let us say, the most progressive work in virology was being done in England or elsewhere in the world at the time. This was approved by the National Research Council and I arrived at the Lister Institute the day before Christmas, December 1933.

Dr. Benison: Did you know anyone?

Dr. Sabin: Yes, I knew someone because during the period of my internship at Bellevue Hospital there was a visiting resident on pediatrics, a young Englishman by the name of Donald Bakeham, with whom I became very good friends during his stay in New York. He was a very wonderful person. We had interesting relations later on, and as a matter of fact, he met me on arrival. It was the day before Christmas and he had to leave London to be with his family. He was still a bachelor at the time, and there
I was in a small hotel (I think it was the Brown Hotel somewhere off Oxford Street in a rainy, snowy sludge... it was just a penetrating cold about as miserable a situation to arrive to that I can imagine.) I got off the morotania traveling third class, but on arrival I found an invitation from John Leddingham, the Director of the Lister Institute to come to join the family the next day for Christmas dinner. And this was a very heartwarming reception. And there I was, not only with his family (his home was on the top floor... it was right in the Institute itself), but there were a number of the members of the Institute I met for the first time and I must say there was a wonderful feeling of warmth that was immediately developed on that Christmas Day, 1933. John Leddingham was a very kind man and I met a number of the people who worked there. I don't remember all of them who were there, but at any rate, many of the important members of the Institute. So that was the beginning.

Dr. Benison: That was your introduction.

Dr. Sabin: That was my introduction to the Lister Institute, so the next job was to find a place within walking distance... a place to live. And the Lister Institute is on the enbankment of the Thames, so I went looking for places to live. And during that period between Christmas and New Year's, I found a place on Oakley Street for bed and breakfast, with a gas heater. It was the sort of thing where you feel warm in front and freezing in the back. But that was my first place.

But, let me interject this here, because a very odd thing happened during that week between Christmas and New Year's. I discovered through mutual friends and also
correspondence that Dr. Bredner's wife had just come back from a trip abroad. She was getting over the death of her husband and she was staying with friends in London. We met, and as a matter of fact, she invited me to a New Year's Eve party with her friends. So we had reunion. What might have been a very happy occasion was, of course, spoiled by the fact that seeing her, I had the memory of Bredner...my colleague and associate, the source of the B-virus, and I couldn't help but constantly think of the sad days that I was with him as he was dying. In one sense, I was not alone and in another sense, it was a mixed feeling and I became established there, but very soon thereafter, I gave up this unpleasant life in the gas heated room, and my friend, Dr. Donald Bakeham, who was then a bachelor and I decided to try and find a furnished apartment that we could share because neither one of us could afford to have one alone. And quite by accident and luck, on Glee Place, which is a place where some of the most beautiful studios in Chelsea, there was an author's studio...a lady was going off to Australia, and we rented that. That's where I lived until Donald got married, so I was settled in this beautiful studio and walked every day, rain or shine, to the Lister Institute.

Dr. Benison: Were you formally assigned by Leddington to work with someone or did you choose to work alone?

Dr. Sabin: I was asked what I wanted to do. And I said, of course, I brought with me a virus. At that time, I called it Bredner virus because the editor of the Journal of Experimental Medicine, Peyton Rous, had not yet abbreviated it to B-virus.
What I said I could do with it by the time I left New York during my internship was very little and I would very much like to be able to pursue further studies on it. The monkies that we had available in New York at the time seemed to be resistant to it. I wanted to try again. Did they have any monkies here? After all, he was bitten by a monkey and my hypothesis seems to be that it may have come from the monkies, so it was necessary to do that. They said all right. I said, very frankly, Dr. Leddingham, before I left, Dr. Rivers indicated that he was very much intrigued by the reports of Eagles from your Institute, that the vaccinia virus can be grown in a self-free media and I regard this also myself as a very important question for virology. So before I get involved with the B-virus that I brought, I wonder if I could learn from Dr. Eagles how to grow the vaccinia virus in a medium without living cells. So he said very well. There's no room in the lab which Dr. Eagle's works, but there's room in the laboratory where Dr. Aimes works. Dr. Aimes, he said, was working with vaccinia and he's using the ultracentrifuge we have, which has the capacity of sedimenting the vaccinia virus and we can prepare purified elementary bodies of vaccinia. He said, why don't you go to work and get yourself a bench in Aimes laboratory. Aimes was a very fine person with whom I developed a friendship over the years, and from him, incidentally, I was trying to learn the use of this ultracentrifuge and purification of virus and sedimentation.

Dr. Bension: Was it a very large machine at that time?

Dr. Sabin: Actually, it was down in the basement. All it had was 14,000 revolutions per minute by comparison with the ultracentrifuge models used in virology that were
developed later, it was a very primitive thing. But it was certainly better than anything that was available in the United States at the time. Nobody was using the centrifuge with that capacity, with that gravity. So I was very glad to learn the technology involved with this and at the same time I began to observe what Dr. Eagles was doing. And, actually, the first weeks were spent in trying to determine Dr. Eagles. Now, I was not an expert virologist, certainly, but I had worked already for a number of years (from 1926 to almost six or seven years) in the laboratory, and the first thing that impressed me with Dr. Eagles was doing was the way he was titrating virus. There were two questions basically that had to be resolved in this very important issue for virologists. For the most part, Dr. Rivers was really the father of American virology; he was the forerunner at the time. The main thing he was thinking about, because he couldn't possibly imagine another possibility, was to make sure his medium was really free of living cells. Well, the way he disintegrated the cells and the way he centrifuged out whole cells, the probability that an occasional cell may be left behind, perhaps could not be eliminated, but the amount of virus that it would yield would be such that it would be hardly an important factor for any significance in serial propagation. But the moment I saw Dr. Eagles work, and I'm really sorry to say that because he's really a very nice person, but he used a horrible technique for titrating virus. It was contrary to good discipline technique. Now in order to understand what is involved here, you must understand that in titrating of virus, you make dilutions of the original concentration of the material. And for this the discipline procedure is to use a separate pipette for each solution. In other words, you take a certain amount out, whether it is 0.2 ml. or 0.5 ml. with a pipette. You put it in diluting fluid and then you shake that up. You throw away this
pipette so you don't carry anything over. You start with a new pipette and so you make serial dilutions out to million or ten million or whatever. In this way, if you find that the virus, let's say has a tita of one million infectious units as you determine by measuring it ultimately on the skin of a rabbit because there were no tissue cultures at the time to measure the concentration of virus, and then if you inoculate that in a medium and it undergoes a one to a hundred dilution and you carry another, and you find out that it has undergone a dilution beyond one in a million, and you still have virus there, perhaps ten thousand or a hundred thousand, obviously, it must have been propagated the nova so the real issue was the nature and means of determining the concentration of the virus. But where Dr. Eagles was doing, and apparently, I wouldn't say everybody in England was doing it at that time, but you know we're used to having pipettes these days...it's nothing to have a lot of pipettes. He used a capilary. He would draw out a certain amount of capilary and transfer it from the original specimen to another and then he would wash it out, wouldn't even change capillaries, and the same capilary he then used for making the next dilution. And as I watched it, I could see that the first time that he could capture some virus up above which was not washed out. At any rate, it was a totally unsatisfactory method of titration.

Now to get another clue as to whether this could be the real factor other than some residual living cells, I look at the results of histitration......how it came out in dilutions and it was very uneven. And to even my very unsophisticated state of development at the time, it immediately suggested that now he had it, now he didn't, and it was probably an accident. This was my suspicion or whether he happened to pick up a bit of virus that remained in the capilary that he didn't wash out as he was carrying it from the most concentrated. Well, I was very depressed because I don't
like to find situations or a colleague working at a respected institute, but finally I felt there was only one way to resolve this. Because I was immediately very critical of his work and I said, let's set up an experiment from the beginning. You set it up. You take your self free medium, extract the cells, and I think at that time he used a sticular extract and rabbit testicle extract and you innoculated with the original innoculum, which let's say has one to a hundred thousand or a million infectious units, and then at each passage you do the titration your way and I'll put some of the same material and I'll do the titration my way using separate pipettes in the discipline manner to which I am accustomed to working. Now, if we get the same results, find. But, if according to your results you conclude that you have gotten propagation according to my results shows that the virus has disappeared, then that's as far as I'll go. Well, it turned out that that was the clue to the report, that when I used separate pipettes the titrate in the proper manner, there was no multiplication of the self free medium. So within a really very short time, I was able to write. He say it and he couldn't refute the thing because it was a simple technique.

Dr. Benison: What was his reaction?

Dr. Sabin: Well, he was stunned. But he was a rather peculiar person. Actually he was a Canadian working at the Lister Institute. A very nice person, you see, socially, very sophisticated, cultivated, but as a virologist, he was absolutely an inentity. And so, I stopped any further work; I mean he was obviously hurt because this was
published and I didn't want to publish it. I didn't publish it. I don't like to do that. I was a guest there. I thought it was up to him, and I don't think he ever did. I don't think he ever retracted. As far as I remember, it's still in the literature; he never retracted it, but I wrote a letter: "Dear Dr. Rivers: (I didn't call him Tom at that time yet... I was nobody still) Here is the answer to the riddle you asked me to resolve at the Lister Institute (and I think it was two or three weeks) and it's all done. I'm going to work on other things." I don't know if I have the correspondence, but Tom Rivers was really elated. I think he went around telling friends. You see, Sabin solved this in less than a month. I always knew that this wasn't possible. I mean he was really elated. This was the playback that I got.

Dr. Benison: There are a number of things...work with tissue cultures in Lister Institute, really especially tissue cultures as applied to virology, __________ (incomplete sentence).

Dr. Sabin: No, it wasn't yet a developed art. Tissue culture the way we understand it now, is not the way it was done then. There were several ways of doing things then. At that time, you either minced up some cells and you had them in a fluid that would support their survival and you could put in a virus and as long as the cells survived, you could show that in the presence of the living cells there was multiplication as measured in an animal. Because the tissue culture techniques came later when John Envers developed things in a more sophisticated manner after 15 or more years later. So the difference was the issue not the tissue culture. What Eagles was doing was instead of putting little minced up bits of surviving tissue, he just ground them up,
centrifuged out the things that might have unground cells or frozen or thawed; I forget the details, and then he had a supernate which had no living cells, no intact cells, so the issue was not the kind of tissue culture; it wasn't that he used a bad tissue culture, it was just plain bad technique. And this is something that I have had to encounter the rest of my life...even now. The ________________....

I'll never forget that...bad technique gives bad results. Careless undisciplined work in the laboratory gives unreliable, unreproducible results. This was my introduction and nobody taught me that; I had to teach that myself and when I arrived at the Lister Institute, I didn't learn that from them...I had to teach this investigator. Now why somebody at the Lister Institute, whether its a man like Leddingham or whether it's a man like Aimes, did not have the guts to say now this is a revolutionary statement before I let this publish, I want to see what this man is doing, why nobody at the Lister Institute did what I did in one week, to me is an unpardonable way of doing things...freedom of research, autonomy of the investigator has certain limitations.

Dr. Benison: Could it be that they really didn't believe Rivers when he said that a virus is an obligate parasite that could only be...

Dr. Sabin: No, nobody ever checked on him. Nobody ever checked on Eagles. It was not proper. You don't do that to a colleague.

Dr. Benison: But now you had checked on him. What was the reaction to you?
Dr. Sabin: Well, the reaction to me did not change at all the good relations that I had with my other colleagues. They were perhaps scientists, like everybody else. I wasn't happy about having discovered that, but I'm not at all sure that other people at the Lister Institute were unhappy about the fact that Eagles committed such a horrible error.

Dr. Benison: One thing about working in England at this time presents a problem that you don't find in America. Almost the first thing that you have to do when you came to England was to apply to the Secretary of State for a permit to work with animals.

Dr. Sabin: Well, this is a formality which the office handled and in which no one stood in the way of progress, since perhaps unnecessary red tape, but that's the way they worked it at the time. In the United States we have other kinds of red tape and you learn to live with it. It presented no problems at all.

Dr. Benison: Well, in a sense you still had room in Aimes room.

Dr. Sabin: So now came the issue that I am finished with the vaccinia because Aimes lab was the place where vaccinia work was done. You don't want to mix up viruses in the same room so there were two things that came to mind. I said, I really want to work out this B-virus. It so happened that there was a wonderful experimental neuropathologist who was also a virologist at the Lister Institute at that time. His name was E. Westenhurst. I think the E. stood for Edward, but everybody called
him Westenhurst. And he was originally a neuropathologist, very well trained, and he began to study some years before I came to the Lister Institute viruses that attacked the nervous system. He became very much interested, for example, in the virus of sheep called louping ill, and because it produced very interesting lesions in the cerebellum and so on, and then actually he continued at the Rockefeller Institute at Princeton the year before I came. And during the course of this work, he developed a facial paralysis that remained...he himself became infected with louping ill. Louping ill infects human beings it was found out later. And he had a laboratory at the Lister Institute, a laboratory with excellent facilities for cutting the most beautiful sections and getting the best kind of histological material for the study of the effects of viruses because in those days, you studied viruses by what they did to susceptible hosts and, therefore, histopathology constituted a terribly important aspect of it. Tom Rivers himself was an expert histopathologist because the sections he would get on anything he would study, whether it was chicken pox or virus-3 or other things, were absolutely perfection. Well, this was the situation with Westenhurst. So it was decided that I should move from Aimes laboratory to Westenhurst's laboratory. And it was there that I began my studies on B-virus with a very fortunate accident. Westenhurst told me that he happened to have 8 monkies, because he had done some very important work on polio, incidentally. Incidentally, I think another reason that influenced my going to the Lister, I remember now, was that Westenhurst and I think Fairbrother had done some very important work on polio supported by a commission by which Dr. Park was president and since I had begun working on polio in 1931, I think the idea also was that I would learn something more about polio research there. But I never did any polio research. Well, the thing was that I then
transferred to Westenhurt's laboratory and it turned out that he had 8 monkies and I
decided to test their serum to see whether they would neutralize the B-virus. Now
the few monkies I was able to work on in New York all neutralized this virus. This
is what gave me the idea that maybe monkies were carrying it and I couldn't do any
experiments on it. But it turned out that 7 of his 8 monkies did not neutralize
this virus, so apparently they had not been exposed to it, and we were then able to
go ahead and do experiments to see what this virus does in monkies. And this became
an important joint study. I learned a tremendous amount about histo-neuropathology
and the proper histopathological techniques, but at the same time, I was carrying out
serologic work because now that I was able to infect monkies and produce anti-B-virus
serum, I could then use experimentally produced sera studied against Herpes and I
became involved with the whole series of studies which then became the basis of those
publications. But while I was doing this, I somehow or another (I don't know whether
I had any spare time on my hands, but I wanted to take advantage of what was present
at the Lister Institute), I could not forget that ultracentrifuge down in the cellar.
So I decided to see whether or not, for example, these viruses with which I was
working, B-virus, Herpes, pseudo-rabies, whether they would also be sedimented in that
centrifuge the same way the vaccinia was. Because after all, vaccinia was a larger
virus than Herpes simplex and B-virus and pseudo rabies. Well, they were sedimented
and as a result of that I then began to plan a series of studies to determine what
happens to the virus. I mean, what can I find out about the virus in a mixture with
an immune serum, with an anti-serum which renders the virus inactive on innoculation
in animals. And this is what led to my studies on various aspects of immunity to this
particular group of viruses, large viruses, that could be sedimented by the ultra-
centrifuge during my stay at the Lister Institute. So you see how one accident leads
to another. The request by Rivers to find out what's wrong...how to cope with a virus in the self-free medium, my bringing over the B-virus to carry out further studies and encountering a very wonderful person in Dr. Westenhurst, who was very helpful to me, particularly in the experimental pathology of rabbits and monkeys, and taking advantage of the one thing virologically that was unique in the Lister Institute...I think other laboratories in England also had this...was developed in Britain, to study the effects on the virus in a neutralized virus in a ___________ serum mixture. (incomplete sentence).

Dr. Benison: I want to go back to the B-virus studies, Dr. Sabin. One of the things that almost seems pro forma is, you say, I got some pseudo-rabies anti-sera from Schoke. I got virus-3 from Andrews. Now this giving of material is part of the research process. Do people willingly give up material?

Dr. Sabin: No. I wouldn't say "giving". It's exchange. Now, why? In the first place, the question is asked, "Why should I bother Schoke for pseudo-rabies virus which is a virus in pigs and cattle, and why should I bother Andrews for virus-3? The reason is this: that the pseudo-rabies virus, although it had distinctive properties, had a histopathologic manifestation that was very similar to the Herpes virus and to the B-virus. It produced a certain kind of intranuclear inclusions ___________ ___________ (incomplete sentence). During the year that Andrews spent at the Rockefeller Hospital working with Tom Rivers before, looking for virus of chicken pox, they came across (and I think Andrews was probably the senior author if I'm not mistaken) a virus after inoculating certain rabbits with chicken pox material. It
also produced similar inclusions in the nucleus itself, similar to the Herpes simplex and so on. And then it turned out to be they reached the decision that it was a spontaneous virus in rabbits. So here he had pigs and cattle, monkeis and man and rabbits, and what I was thinking at the time, and I think this was perhaps avant garde, a revolutionary concept that a virus with the properties of Herpes simplex in man, it existed in a different, though related form, in other animals...in the rabbit, in the pig, and actually as you learned later, it is transmitted from the pig to cattle with the ______ host as the pig, and then monkeis had another one that was more closely related to that of man. (incomplete sentence) And the gist is that animals, different mammals, were evolving so the viruses were part of their heritage were also evolving. So I had to have material to determine their relationships serologically and what pathological differences there were. And that's why I turned to these people. And this is a constant part of the scientific interchange among investigators. As a matter of fact, when an investigator reports on a virus or some reagent publishes and when he refuses to give it to another investigator, that's a very black mark against him. Now that happens, but it immediately marks that man as someone who is not following the principles of a scientific endeavor.

Dr. Benison: Now did you know Iva Schope or Andrews when you asked for the virus-3 and pseudo-rabies serums.

Dr. Sabin: I knew Schope as a result of my visits to the Rockefeller Institute, although he worked out of Princeton during my years of internship at Bellevue Hospital. I used to visit the Institute so I knew Schope well enough to ask him for the virus
and for some serum, which he sent me. Andrews actually I met only after my arrival in England because I didn't meet him during his years at the Rockefeller Institute in New York and Andrews at that time, together with Wilson-Smith, or was it Douglas... the name escapes me now... had just transmitted influenza virus to _____________ (incomplete sentence). And it was a big discovery. It opened up the whole field of influenza virus research. And the National Institute for Medical Research in Hemstead was really a place at that time where some of the most advanced and exciting work in virology was going on. They were working with influenza virus by totally new methods. Alfert had developed the membranes by which one could then show that not all viruses were the same size, that there was some rhyme and reason and regularity that a given virus that a given size that this was part of its characteristic and there are a number of very good people in virology. So I used to visit, although I worked around the clock almost at the Lister, the Hemstead National Institute for Medical Research and that's when I came....

Dr. Benison: Was Samuel Betson one of that group?

Dr. Sabin: Samuel Betson was elsewhere, but the society in London that had quite a number of virologists. I think the Society of Microbiology and Pathology, I think, numbered only 70 in all of London. But they used to meet in different places once a month or so, and I used to go to those meetings, so I met the people. And Betson was the man, the forerunner so to speak, of elementary bodies at the time because he was interested in coma... this was actually followed later, but other elementary bodies.
It turned out to be what are now called bitsonia, you see, because they were not really viruses. And then there used to be the Conversetcioni of the Royal Society. In that way, I got to know the people who were really working in virology and microbiology in London and that was a very, very gratifying and stimulating experience, and I think for a young man that is a very important part of his career development.

De. Benison: Now, Dr. Sabin, if there were the kind of debates that grew up between Eagles and Rivers, there was another debate that grew up about immunity to viruses. Would you tell me what the basic theories at the time about immunity to viruses, and the reason I raise this is because you, too, become interested in this problem of the process of immunity.

Dr. Sabin: As far as I remember, I wouldn't say there was a debate in the sense that there was one group of investigators who believed that immunity to viruses was one thing and another group believed that it was another. People had for several years been attempting to explore the mechanism of immunity to viruses. They wanted to know, for example is it like antitoxic immunity or is it like certain kinds of antibacterial immunity, although there are different kinds of antibacterial immunity. And, therefore, there were issues that people were trying to resolve. If its like antitoxic immunity, then the anti-serum should combine with the toxin and neutralize it and that's finished. It it's like a certain kind of antibacterial immunity, then there was a question of whether or not an antibody against the virus combined with the virus, but in order to make the virus ineffective, it would have to be taken up by _________ and destroyed (incomplete sentence). In other words, the process of opsinization, so to
speak were bacteria that are coated by antibody are taken up by polynucle lucasides and then destroyed. So that there were many experiments that had been done, and among them there were experiments by Andrews and others, that had approached this question. There had been studies also carried out at the time with bacteriophage, which was beginning to move forward. This was a field in which Burnett had made some early at starts, too, and Burnett, incidentally, was the National Institute of Medical Research just the year before I arrived. He was there in 1933 and returned to Australia when I arrived. So there were different approaches attempting to find out what was going on. In my previous reference to Burnett and bacteriophage, I think now I should have said Alfred and Andrews had worked on the neutralization of bacteriophage. I don't have any real recollection of Burnett's having done it. Now the issues which I tried to clarify were actually an outgrowth of observations published mostly by Andrews and some of the English workers before. And they were that there was no evidence for any certainly firm union between virus and specific protective antibody; we're talking about the large viruses now. And also the peculiar phenomenon which was actually first described by Andrews in 1928 and by Todd and Bentley in 1928 who observed that mixtures of vaccinia and immune serum which were innocuous for the skin were nevertheless infective when injected in intrasterticularly intracriberially, or intravenously, and also in the case of Andrews, that skin inactive mixtures of virus-3 and the corresponding immune serum were likewise infective when injected intrasterticularly or intravenously. Todd found that mixtures of foul plague virus and immune serum which were inactive when injected intramuscularly were infective when injected intravenously. And recently, at that time, I had observed in 1933 the same phenomenon with B-virus. The B-virus immune serum which failed to prevent infection when injected
with minimal quantities of the virus intracerebrally while it protected very well by the intracataneous route. Now the tools that I used were a) my knowledge of the three viruses that I had been working with at the time; namely B-virus, pseudo rabies and vaccinia (and I used vaccinia because Andrews had done so much work with it before) and b) the ultracentrifuge. Now I tried to determine whether or not the neutralization of these viruses was in any way similar to either a toxin anti toxin reaction or to a reaction between a bacterium and an antibody with requires, let's say, a phagocytic cell to destroy it. Now the phagocytic cells were now necessary for certain viruses that had already been demonstrated previously, so that was not the crux of the issue. My main objective was to see whether unlike toxin anti toxin reactions which really become irreversible after a time, might be comparable to the virus antibody reactions... that if the mixtures of virus and antibody were kept long enough, that ultimately it would be an irreversible union. And my experiments definitely showed that that was not the case. That no matter how long you kept the virus and the antibody together, and if I subsequently using the centrifuge to sediment out the virus which came out quantitatively, I could recover a great deal of the virus. And the real issue, as I look at the data, and as I know Andrews looked at them after the data were published in 1935, was whether my experiments demonstrated that all of the virus was recovered or whether they demonstrated only that 10-20% of the virus remained active even though the mixture was completely inactive. Well, I must say that there are justifiable grounds now because the methods that we used for quantitating virus do not permit a clear answer. On the other hand, I think I was justified in saying that the data did indicate that in a mixture of virus and antibody, which was completely non-infectious at least by the technique used, that large amount of free virus could be demonstrated by centrifuging the virus away from it. And that therefore, I could say
that the complete inactivation of the virus did not occur in a mixture that was no longer infectious. Perhaps my words that there was no evidence for union between the virus and the antibody was not justified by the data presented, but the fact that the virus was not destroyed by the antibody, I think that still remains. So there were other approaches that had to be made and I will not go into the details of every experiment, but the most striking one was the extension of the studies which I mentioned a little while ago, particularly recorded in 1928, by Andrews and by Todd earlier, of the peculiar difference in the ability to demonstrate neutralization of the virus when different tissues of the same animal were used or different animals were being used. And in this study, I was definitely able to show very carefully, it was very quantitative, and this is especially with B-virus which was a virus, the smallest amount of which injected into the skin was capable of producing paralysis and death. In the same way, the amount was exactly the same as when injected directly in the brain, and yet mixture of serum and virus regardless of the proportions which were nicely inactive, in other words the virus was neutralized when the mixtures were given into the skin and the animals were protected, not only was the skin lesion prevented, there was no paralysis. The animal didn't die; the animals remained well. Yet when the same mixtures of minimal amounts of virus with large amount of serum were given to the brain, there was never any protection at all. And the same similar phenomena were observed in experiments that I made with pseudo-rabies in guinea pigs, using the intranasal and the intracerebral route and using similar mixtures in rabbits subcutaneously. I think all of these experiments that I had done with Herpes and that showed exactly the same thing...that mixtures of minimal amounts of antibody with large amounts of virus, which were inactive when given in the skin; yet when inoculated into the brain, there was no neutralization. Now a variety of orienting studies that indicated that it had nothing to do with the sensitivity of one tissue versus another, that one was not able to detect smaller amounts of virus than the other, that it had little to do or nothing to do with the diffusion in the inoculated side so
that you could think well, the serum could diffuse faster and so a sort of mass law type phenomenon could enter into it where the serum was being separated away from the virus and the virus became free to infect because using disticular extracts in the skin because of a factor that produces edema swelling and this rapid diffusion in such areas that didn't make any difference. It was inescapable--that the tissue determined whether or not a given mixture of serum and virus would be infective or would not be infective. That it was not just a matter of incubating virus for a long time, that the serum itself could inactivate the virus. There was no evidence in these experiments, and when I think back now with the more sophisticated procedures, I still think that the experiments have not been done that would show that a virus is definitely inactivated, that the mechanisms of neutralization occurs in the absence of the particular tissue in which the phenomenon is to take place. And for that reason, I find it very interesting to read the conclusion that I reached in 1934, which was published early in 1935 in the British Journal of Experimental Pathology. Based then on these studies are the different capacity of different tissues to influence the outcome, the inactivity of a given serum virus mixture, which I said that the studying of these data is difficulty to escape the conclusion which was arrived at in the invitro experiments of the preceding investigation that I reported myself, that the mechanism of immunity to these viruses--I'm not generalizing here, I say to these viruses--these are large viruses, its Herpes, its B-virus, its pseudo-rabies, its vaccinia--its these viruses, is intimately bound up with the cells to be protected. I say, "One cannot readily correlate these findings with any hypothesis which assumes that protection is the result of the direct action of the immune serum on the virus. It appears to be quite definite that ________
given tissues of equal susceptibility and equal amounts of immune serum in virus, the outcome is not the same. The mechanisms whereby one tissue is readily protected, while another is not, is quite obscure, and its illucidation is of the utmost importance to clear understanding of the nature of immunity to filterable viruses". This conclusion I reached 40 years ago and it was published early in 1935, and when I think back of the studies that have been made in the forty years subsequent, we still do not have an explanation for this phenomenon. And I think it is extraordinarily interesting to visualize what it is that occurs at the cells surface when a virus becomes ineffective in the presence of immune serum because that certainly is a fact. And, also, it is something that happens very fast. Because if you give the virus first and you just delay the introduction of the serum by a few minutes, it's too late. And I think that one must investigate the possibilities that both the antibody may have certain different groupings, one of which goes for the receptor (of part of the receptor) of the virus and another for the virus itself. I think at the present time, I would not maintain that there is no interaction between virus and antibody, but I would rather still maintain and stress the need for investigation, that the inactivation of the virus is not the mere consequence of a union between the virus and the antibody, because that cannot explain why it will happen in one tissue and not in another tissue and that it may very well be that the receptor substances which must combine with the virus before the virus can start its effect on the cell, they somehow also be acted upon by some component in the specific antibody.

Dr. Benison: Dr. Sabin, I have a number of questions. First question is, as you look back at these experiments, what were the crudities that you see? What were
the things that you did then that you would not do now?

Dr. Sabin: I would certainly use better quantitative methods for determining the amount of virus that could be recovered.

Dr. Benison: Were there other methods to titration at that time?

Dr. Sabin: No, at that time, no. Okay, I could have used instead of 20 rabbits, 200 rabbits, which would be an impossibility. I wouldn't be able to get them from the Lister Institute, I wouldn't have the cages; it would be impossible. So that at that time the techniques which make it possible accurately to determine the concentration of the infectious virus were not available and they didn't become available for another generation or so. So, now it could be done. And I think it would still be interesting to see just what happens. Take these same viruses. Take the anti-serum against them and not all anti-sera are the same because anti-sera obtained at different times of antibodies against different components of the virus. I think we are dealing here chiefly with the antibody against the components in the coat of the virus particle which has to combine with a receptor substances of the susceptible cell, and then see what happens when you centrifuge the virus out and you re-suspend it, and you titer it quantitatively.

Dr. Benison: Newspapers. There are a series of four or five papers on problems of immunity. It's interesting that the work of Andrews leads you into an examination of problems of immunity and one of your critics of your work at the time is Andrews. Do you remember the criticisms which he made?

Dr. Sabin: He never did it publicly, but there was a correspondence between us because obviously what I was doing was very much influenced by what he had done
four years before me. And as regards the first part of my work using centrifugation to separate virus from serum, in the first letter that he wrote me, he came out very strongly and said he felt I was all wrong. And he said that in effect (I don't think these data were ever published) that he was increasingly convinced that I was all wrong. And then he proceeded to tell me (and I don't think I have ever seen this published) that he did some additional experiments along with Alfred to check up on the results of centrifugation of serum/virus mixtures that I had reported, but they used a different procedure. They took mixtures of vaccinia virus and anti-serum and held them in contact for one hour at 37 and then washed them on a membrane that would hold back the virus, but would allow the anti-body to go through. And then he said that they first showed that after four or five washings, the residue contained insufficient anti-body to affect the take of one minimal infectious dose of the virus so that they had washed it out quite thoroughly. Then in a series of experiments on 19 rabbits, they compared the titer of the washed virus normal serum mixture and the washing virus immune serum mixture. Now this is what remained on the membrane, the ultra membranes. In one instance in which the unwashed virus immune serum mixture proved that neutralization was incomplete, the virus immune serum mixture went to the same titer as the control. In other words, even though there was some neutralization, you couldn't find that the virus was neutralized. Now on six occasions it went to 1/10th the titer of the control. On eight occasions the difference was more than ten to one. Thinking it possible that the washing technique might give a different result from the spinning method, we used the lab and obtained similar results on four rabbits. They didn't use very
large numbers either, so that their quantitative tests are not any more accurate than the ones that I had used. They didn't use any larger number of rabbits than I did, and again, they obtained similar results than the average difference between virus normal serum deposit and virus immune serum deposits. Now I will grant that by the techniques that we used because quantitatively you couldn't determine between 100%, 80%, 50%, 20% even, that in a mixture that is completely non-infectious, a certain part of the virus can be obtained back. As for example, within a short time after mixture you can do it with toxin-anti-toxins. But with the toxin-anti-toxin mixture, when you allow the reaction to occur for a long enough time, it becomes irreversible. But in all the experiments, at least that I have done in which I have carried the stuff for two weeks, there was no evidence of irreversibility. So let's say that my conclusion that I reached at that time the data did not show that there was any union between these particular viruses and antibody, I would at the present time, if I were a referee, send it back to the author and say that this is not warranted by the data that had been presented. You could say that you could recover large amounts of the virus, but you could not say that there was no union because the reversibility remained. But when you take the whole thing together, particularly with the roll of the tissue, when you can show that the same mixtures using minimal amounts of virus and large amounts of serum, that in one tissue the thing will be infectious and another it will not be infectious regardless of the amount of previous incubation. That creates a situation in which you cannot escape the role of the cell itself, and that's why I still think that there may be something much more complex, much more interesting, than simple union between virus and antibody. I think that the present, more sophisticated techniques where you mix a virus with anti-
serum and you count the number of black forming units; that still does not eliminate the role of something happening at the cell itself. Because you are measuring the effect at the cell surface, and I think it is necessary really to re-investigate the possibility that the neutralization of a virus to the extent that it is influenced, not a virus, certain viruses, let's say, by the cell to which the virus antibody mixture is presented that there may not be a double process in which the antibody does something to a portion of the virus receptor while the virus attaches to another portion of the receptor and it may very well be that the receptors in the brain, or the B-virus in Herpes, are different than the receptors in the skin, and the same way with the other examples that I had provided in which Andrews and Todd, certainly had provided four years before me, leads one to think that there may be something very important. And from what we understand now, something we didn't know before, because whoever thought of virus receptors forty years ago, didn't think of virus receptors. At that time, one was thinking of viruses like little parasites, that they were somehow or another swallowed up by the cell just like a malarial parasite, let's say, and when they got into the cell, they found the right milieu either to multiply or not to multiply. And it depended on what was in the cell. Now we know that there are specific receptors on the surface with which the virus has to combine, just like it is evident that a spermatozoan and an ovum that the specificity of the mouse for a mouse and an elephant for an elephant, also depend on the chemistry being right. You know, the chemistry has to be right. You know, the chemistry has to be right. The prize is much—for spermatozoan and ovum the ____________ as a virus and an acceptable cell, and the way an antibody works, at least for some viruses, especially may very well be involved at the cell surface, which is still incompletely understood. Because
there is no evidence at the present time that would explain that the phenomena
reported by Andrews and Todd in 1928 and by myself early in 1935 on the nature of
the varying protective capacity of anti-viral serum in different tissues in the
same species and the same tissues of different species under conditions where the
minimal infective doses are the same for both. My experiments, incidentally, show
that the availability of complement of components of complement do not play a
role at all, because in vitro tests in which mixtures of serum, that is antibody
and virus, were submitted in large doses to complement, that didn't make any
difference in the recovery. So that I think that while my approach was a little
primitive, I think my questions were sophisticated, while some of my conclusions
I would send back as a referee now, I think there is a basic problem which is
still unresolved and which has not been touched, but you know, some early observ-
ations are very often forgotten and a friend of mine, Michael Sella, of the
Whiteman Institute, in a recent informal talk to a group of scientists at a meeting
of prospectives in virology, I forget now what he quoted, and he said incidentally
the first observation was actually made by somebody else twenty years ago. But
when it was reviewed, the credit for the observation was given to this man, and
he said, I call this murder by review. And the same way people who undertake the
important responsibility to review the literature very often overlook certain ob-
servations or they skim over them very lightly and don't try to go in more deeply.
And, as a result of that, the questions that are raised and have remained un-
answered, I never again picked up because you can't expect the newer generations
to read 100 articles that were published before, so they read a review. And the
only papers they know are the ones in review and so that other work is murdered
by reviewers.
Dr. Benison: You know, when I look back, there are so many things that come to mind. First, Leddingham and Eagles, the ultracentrifuge for their work on vaccinia, yet Andrews and Todd are content with dilution experiments and the membranes. You see the ultracentrifuge and you immediately want to adopt it as part of your method. The ultracentrifuge is there....

Dr. Sabin: Well, actually from the letter that I receive subsequently from Andrews, he did use the ultracentrifuge...

Dr. Benison: ...spinning experiments...

Dr. Sabin: ...after my publication apparently. He went back and did it. But then you see, I had another letter from him in August of 1935 and he said that he wasn't so certain. He would maintain the position that there was some union between virus and antibody that he wasn't quite certain about what the ultimate mechanism what the activation of the virus particle was. He said, "I am in a great difficulty about this antibody business. [August 1935] I feel that I have done a good deal at it and that it is far from being settled yet." It's a little different tone, and "that is from my point of view, highly unsatisfactory". So it was not settled in his mind. "Yet I am at present so deeply involved in other things" that he cannot pursue this further. And he goes on to say something else, "I suggest that the anti-influenza serum may work a bit differently from vaccinia sera. So far, dilution phenomena has not been demonstrable. Also, it is rather strange to get anti-viral serum with demonstrable activity in a one to 10,000 dilution. Dilute __________________ sera also seem to work better when incubated with virus, _________________________, in incubation and being,
of course, adequately controlled. To consider a few points raised in your letter when I said you were 'all wrong', all in quotes was meant to emphasize the wrong, not to be applied to literally."

Dr. Benison: Well, there's another thing. You really begin working with viruses per se in 1931. By 1935 you are dealing with questions apparently on the frontier of virus research. One might say the most difficult questions, immunological questions. And it is a kind of repetity in development--is it because of the time?

Dr. Sabin: Well, perhaps I don't know if that was the intent of your question. When I moved at the end of the one year at the Lister Institute to the Rockefeller Institute, I am trying to think why didn't I continue with these immunity experiments. I think one thing at that time there wasn't such a centrifuge at the Rockefeller Institute. It became available later, but I became very much intrigued about problems of viruses of the central nervous system and I tried to develop certain models which would make it possible to understand better the problems of the epidemiology and the manifestation, the natural history of poliomyelitis. And one of the questions was, of course, in my mind, because polio had already established itself as an on-going issue, was why so many people became infected. We already had that hypothesis then and so few developed paralysis. How the different viruses move about in an animal's body. So not having the proper facilities or some approach to continue with the problems of general virus immunity, I began to work with the various models of different neurotropic viruses and experimental animals, and particularly, utilizing both the technique of measurement of growth of virus, propagation of virus, and the histological approach which I learned
from Westenhurst. Again, you see there is a transition from Westenhurst at the Lister Institute. Now it so happened that one of my technical assistants at Rockefeller was Dr. Peter Olynski; at my disposal was a man by the name of Tyler who used to be Neguchi's technician. And actually I had the laboratory that Neguchi had done all of his work. Well, this technician was a superb histological technician. And he was able to make for me magnificent preparations of cerebral sections of the brain and so on, so I became involved in chasing viruses around the nervous system. For example, one of the discoveries that came early was the difference in behavior of young mice and old mice. And the difference in invasiveness, or the roots of evasion of different neurotropic viruses which I was able to follow by histological studies. And then, of course, I began also the studies on polio. Unfortunately, I say unfortunately because if I had begun my studies on polio somewhere else rather at the Rockefeller Institute, I may not have used Flexna's famous MV strain (Mixed Virus). Because I think that mixed virus strain really possibly, it's hard to say, altered considerably the course of events because it was no modified by continued passage in the brain of monkies that it lost all of its ability to multiply in non-nervous tissues.

Dr. Benison: It became neurotropic.

Dr. Sabin: It can be neurotropic and still have infinity for other tissues. It was strictly neurotropic...strictly is the word. But at the Rockefeller Institute we used Simon Flexna was still around...he used only the strain.

Dr. Benison: I don't want you to jump ahead.
Dr. Sabin: Well, that's the explanation. I was not carrying on, starting on a totally new path of investigation after leaving the Lister and starting at the Rockefeller.

Dr. Benison: The work that you do at the Lister seems so vital that I was puzzled as to the complete change when you come to the Rockefeller. Would it be fair to say in summation that what you got out of the Lister Institute was a new string to your bow and armentarium for work that is the ultracentrifuge. You'd learned histopathological technique from one of the masters and you had made an extraordinary number of friends in the virus community...and I don't means friends in the social sense, but in a sense you were exposed to one of the frontiers of virus research. Because certainly Andrews and Todd and Wilson-Smith were on that frontier.

Dr. Sabin: I would say that the development in my virological expertise during that one year of work in England, I lool upon as invaluable experience. The tremendous aura and climate of activity in England at the time and to be exposed to the thinking of a critical approach and the methodology used by those people, left a very great impact on me. And, of course, I shall be ever grateful to the fact that the director of the Lister Institute put no obstacles in my way. He never limited the number of animals that I used, although I used many more than others were using and gave me an opportunity to do good work; I mean hard work, whether it was good or not is another matter, but it was really an invaluable part of the development of a scientific career and that's why, now, forty years later, I think
that young people who are starting out in their careers should be given opportunities through scientific research fellowships the same way I was given by the National Research Council to work abroad, but not merely as a pair of hands on somebody else's crutch. Go abroad, especially where there are new techniques that would broaden the horizon; let them learn the techniques, but in choosing the people or sending abroad for that type of training, I would be very careful to limit it to those who have already demonstrated a capacity for some sort of independent thinking. If you ask them what they want to do, they can give you an answer. They won't say, I don't know. I'll go and see what I can learn. There on the horizon, they have been reading the literature, they know what's going on, they want to go and work there because...you see. So I think this remains a very important part in the growth and development of the scientific career of an individual and, of course, the situation has changed very much in the last forty years; The United States has become a center and is drawing people here from other countries, and I think we should insist the same way that they send us only people who would be able to take advantage of what they learn and upon returning would have the opportunity to utilize this information.

Dr. Benison: There's one other thing before we close, Dr. Sabin. One can't help but notice in these papers written in the early 30's about virus disease, that the model, the analogical model, from what happens in bacterial disease. This is the jumping off particularly in problems of immunology. When is that model dropped?
Dr. Sabin: Well, it is not unnatural in any scientific pursuit to go from the known to the unknown. And until you know more about the nature of the viruses and you must investigate the extent to which basic phenomena which applies to bacteria and toxins, apply also to viruses. Later on when totally new technology are working with viruses with new conceptions of how they multiply and what they do when they infect the cell, then they change. As soon as it is clear that a virus is not equivalent to a smaller obligate intracellular bacterium, then the whole thing changes. That was an important transition. You'd be surprised how many people at that stage still thought that a virus was nothing more than a much smaller bacterium which had lost the capacity of its own metabolism, had to depend on the cell to produce ____________ and there wasn't even evidence on how those ____________ were assembled, that they might not have been assembled within the wall, ultimately of the bacterium itself the way they're assembled in a large obligate intracellular parasite.

Dr. Benison: Leddingham, himself, had such a belief.

Dr. Sabin: Yes, but, of course, there was the other extreme who liked to look on viruses as purely chemical substances. And that was _______ and Brenner for example, bacteriophage...that it was a very simple chemical substance...its quite different, and then gradually you see as the first virus was really crystallized, I don't know if Stanley would be really one of the subsequent ones, but at any rate, it became evident that you were dealing with a new species of...it's not animal, vegetable or mineral...but it's something else which is a virus...
something that is so ____________, quite different from anything else. Just as more recently now, we have not yet come to grips with it, but we must come to realize that there are such things that are called viroids or agents that produce ____________ disease like ____________ in human beings and they don't have any of the properties of viruses. Yet, it's possible to transmit it, syphilis.

Dr. Benison: So this is really a transition period. You might say a transition period from bacteriology and pathology to something...

Dr. Sabin: Virology is not just small obligate intracellular parasites...its smaller than bacteria. You see and very gradually, of course, as knowledge developed, the separation between viruses and recetsia and recetsia and betsonia, because, of course, there is a great deal of work on citocoses ____________ became only later. Now we spoke of Betson before. He's done quite a lot of work on the elementary bodies of citicosis. Of course, people still talk of citicosis as a virus. It just doesn't have the same properties as virus. That's why its put in a separate group from betsonia and recetsia in a separate group because they don't operate like viruses. And later on, it was necessary to put the ______ pneumonia like organisms in a separate group because even though they have simple reproductive units no larger than, lets say vaccinia virus, they operated quite differently.

Dr. Benison: So this is the period when definitions are being made?

Dr. Sabin: The knowledge was being accumulated to permit logical sub-divisions.

NOTE: Last page from the letter from Andrews, 7 August 1935.