Oral History Interview: Dr. Walter Nelson-Rees

Formerly associated with the Naval Biological Laboratories in Oakland, California, and the development of a cell bank which was initially contracted with Dr. Stewart Madin, which evolved into a facility for both animal and human normal and cancer cell lines.

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Interviewer: Dr. Robert Stevenson, formerly of the National Cancer Institute.

Nelson-Rees: Well, I suppose, as I figure into the picture, it was my having just graduated from Cal and was available after I returned from Germany on a Fulbright. And the laboratory was built to specifications because it was thought that Dr. Madin, with his expertise on the production and utilization of animal cells, could be made to function as not only a repository, and not only a bank for cells, but also a location and a laboratory that could function to ward off contamination or to be able to detect contamination. When I was talking then about a "variety" of cells, we used to refer to it as "Noah's Ark under Glass." We had cells from a variety of animals that were chosen for specific reasons. As has been mentioned in our conversation before we started recording, the natural utilization of aquatic mammals, because of their isolation from terrestrial viruses, for instance, or the utilization of, or attempts to grow, cells from hibernating animals--bear's cells--cells from bears that are known to hibernate. Some other workers have tried insect cells for a variety of different reasons, some of which related to the fact that the cells are grown at room temperature and might be utilized for experiments which relate to the transmission via insect biology.

We dealt with a variety of other domestic animals, at first dogs and cats, and then animals that were of importance in the matter of human

nutrition, cells from cattle, cells from goats, cells from cows, cells from sheep. Then also, as the matter developed, one was trying to locate a relationship, or to identify relationships, between domestic animals and humans in the matter of viral transmission. Was cat sarcoma, or feline sarcoma, related to human sarcoma? Were pets in the household--cats and dogs--in some way related? So, we instantly set out to establish cell lines of feline origin.Then we thought it would be interesting to look perhaps at lion cells, tiger cells. We never actually obtained these, but it was always a relationship to the human, to the animal, to the domestic, to cells that might relate. We also looked into avian cell cultures--budgerigar and its relationship to warts. There were reports that warts--it was known that they were of viral origin--but that these viruses might be grown in cells that came from animals that were not necessarily the animals from which the virus had originally been isolated, but might grow because it was something that had not been known before.

We studied bat cells, and put lots of bats, because of rabies, into culture, and had a variety of bat cells growing, especially here in California, that related to the feral population of chipmunks and animals found in the forest which would relate to potential sources, of a virus not only, but a range of animal cells within which one might grow different kinds of viruses.

Stevenson: Back in the beginning, the program interest that started the whole project had to do with meetings that were held at the National Academy of Sciences under the auspices of the Virus and Rickettsial Study Section of NIH which drew together a large number of scientists who literally complained about the status of cell cultures which were being used by virologists at the time as substrates for their research. And it turned out

that a number of instances were delineated where cell lines had gotten mixed up and there was a lot of contamination, where dog and cat and calf and other existing cell lines were mixed up and thought to be human, and vice versa, and that there was PPLO, or *Mycoplasma*, as we now call it, rampant as infections in these cultures. And the whole idea was to set up some kind of laboratory network where cell lines could be identified as being authentic and then cleaned up and produced in seed quantities so that people would have a reliable source of material.

Nelson-Rees: Yes. These were the Cell Culture Characterization Program, the Cell Culture Characterization Laboratory (CCCL). There was one at Camden under Dr, Lou Coriell, and the other one at Detroit under Dr. Cyril Stulberg and Ward Peterson. And we were the third laboratory. The "mother" laboratory was the American Type Culture Collection which, at that time, was only a very small--

Stevenson: It was just starting.

Nelson-Rees: Just starting. A small laboratory on M Street, I believe it was, in Washington, D.C.

Stevenson: But the idea of the Oakland Lab was to be primarily the gurus on the animal cells.

Nelson-Rees: Animal cells. That's exactly right.

Stevenson: And Dr. Madin, in conjunction with an assistant named Norman Darby, had established several cell lines which were widely used, the calf kidney, the dog kidney, and the pig kidney (MDBK, MDCK, etc.).

Nelson-Rees: Well, originally the Madin-Darby canine kidney cell line and the Madin-Darby bovine kidney cell lines had been utilized by Madin in his laboratory as a veterinary vaccine base. And, from there, then grew these large numbers of other animal cells which we sought: aquatic mammals

which we obtained from Florida, or from institutions in Southern California. We then established a laboratory, as Bob Stevenson indicates, to characterize--it was a matter of utilizing known procedures that we could apply to the characterization of cell lines and cell cultures--and initiate them. And, by that I mean that we did not necessarily discover how to grow viruses in cells. This was known. But we utilized the fact that certain viruses grew only in primate cells. Ergo, if a cell had been contaminated with mouse cells, or with rat cells, or with rabbit cells, it would not show a cell cytopathic effect (CPE).

Stevenson: With certain kinds of viruses.

Nelson-Rees: With certain kinds of viruses, poliovirus for instance. But this was a characterization study for us.

Stevenson: And you were hired because you sorted out chromosomes, right?

Nelson-Rees: I had never seen a human chromosome until the laboratory had begun. I had worked with mealy bugs, I had worked earlier on with *Tradescantia palliodosa*, which is a plant; but I was fascinated with chromosome work early on and, when I came back from a Fullbright in Europe, I was hired to add to the armamentarium of likely tests which could be utilized for the characterization of animal cells.

Stevenson: At that time how many chromosomes were thought to be in the human genome?

Nelson-Rees: It was just--Levan and Tjio had just described the human chromosome number as 46. Earlier on, it had still been 48. And many of our earlier papers and earlier textbooks carried the number as, the diploid number, as being 48.

Stevenson: Who, would you say is responsible for the concept that one could, in fact, sort out cell origins based upon specific chromosome numbers and

morphology?

Nelson-Rees: I don't know the answer to that at the moment. I cannot remember who--

Stevenson: Well, T.C. Hsu-

Nelson-Rees: T.C. Hsu, I was going to say, was the guru of--

Stevenson: And then there was Ernie Chu down in Oak Ridge. Right?

Nelson-Rees: But there were people in Coriell's laboratory who had already been doing that work.

Stevenson: What was that man's name?

Nelson-Rees: Green. Arthur Green.

Stevenson: No. Before him. The Frenchman that was there.

Nelson-Rees: Yes. He worked with mammary tumor cells, mammalian cells, and I can't think of his name right now. Maybe I will in a little while. He was very much involved with cells from human breast and human breast cancer cells. Lasfargues, Etienne Lasfargues, I believe, was the gentleman's name.And there was another gentleman, Dr. Warren Nichols, who was cooperating with Albert Levan in Sweden, and they would exchange laboratories. One would go to Sweden and one would come to America on alternate years. He was very much involved with the early-on description of the human karyotype.

Stevenson: And Tjio was the man at NIH?

Nelson-Rees: At NIH. That's right, and Levan. At any rate, it was not just necessarily growth patterns and chromosome analysis and whether a cell was male or female but, sooner than not, we were able to utilize specific staining, pattern staining, staining characteristics and staining methods, in order to identify aberrant chromosomes which could serve as markers for individual cells.

Stevenson: But that was quite a bit later, wasn't it?

Nelson-Rees: That was quite a bit later, but it began by just the discovery that all cells that were examined that were, for instance, human were not normal cells, that they were aberrant cells. Polyploidy itself was early on talked about and discovered and suspected as being an index for aberrations in aberrant cells, but it could also be utilized to distinguish certain cells from each other. Eventually it became strictly--somewhat later, I believe--the laboratory itself, when it was begun, was originally supported, in 1960 and we actually began to work in 1961. And it wasn't until 1965, I believe, or '66, that the Virus Cancer Program developed under Dr. Robert Huebner and proceeded to be governed more by Dr. Huebner's program to examine cells of human origin. And there it was a plethora, a veritable--a daily--requirement, a daily new requirement, of what cells were to be cultured. We had cells from males and females. We had cells from young people, we had cells from old people, we had cells from American Indians, or Native Americans. We were to grow cells specifically from certain black populations. We were to grow cells--these were all normal cells--we were supposed to grow cells, and we did successfully grow cells, from embryonic tissue. Spontaneous abortions were of particular interest. Also from reduction mammoplasty; cells from normal breast and cells from abnormal, but non-cancerous breasts; the normal and aberrant cells from the same individual. Various age groups were of concern. I remember Dr. Huebner once suggesting that perhaps, in order to get cells that were rarely, or never, studied, to place an individual, say, in Yellow Cab taxi stations to be on call and to be there when someone was called during the middle of the night to come and fetch a woman who was in labor and who would have a spontaneous abortion in order to sample--get samples--from a fetus or an embryo, the

cells which would never otherwise be cultured, because he thought that perhaps a cell culture from an individual entity that never came to term might be of value, significant value, in the Virus Cancer Program. We studied cells from just any number of different categories of individuals.

Stevenson: What, if any, feedback did you get from them about the uses to which these things were put, and did they have any significance or contribute to his research results?

Nelson-Rees: As I recall, in a good many instances, there were people specifically studying spleen, specifically studying liver, specifically studying cells from the prostate, specific breast cells, cells that had been obtained through expression of the human breast, cells that had been successfully grown in our laboratory from normal milk and from milk from cancer patients. All of these were distributed and were asked for and were sometimes directly ordered by laboratories for the specific purpose of the study of certain organs and of tumors, or cancer, in specific organs. So, it was an "on call" situation. It was a specifically directed, guided, effort to grow cells from a specific--

Stevenson: But did they ever tell you that these were useful, or did they have--

Nelson-Rees: In many instances there was no other resource, there was no other place that people could turn for these particular cells. And publications ensued.

Stevenson: What I'm trying to establish was whether you had any scientific feedback that enabled you to either modify or improve your own program, or whether it was just simply that they said, "Thanks for the cells," and that was it. You never knew what happened to them, or whether they contributed anything. For example, Huebner came up with the oncogene

theory, and he and George Todaro are given a great deal of credit for recognizing that there are things integrated into the human genome which, if appropriately turned on, will result in cancer cells and, likewise, these things exist in other animal species. Did any of the material that you supplied contribute to those results and those findings?

Nelson-Rees: Oh, I cannot help but think that they did. And I suppose materials and methods in their publications many, many times referred to cells obtained from the Laboratory of... and that was our laboratory. In other instances, we were directly involved with growing cells with certain chemicals, with mixing different kinds of cells and making different known kinds of cells growing together in a symbiotic sort of relationship in the assumption that while cells were growing together and dividing together that there might be interaction between these cells at the cell culture level. And the results, in some instances, were very promising. I can't cite any specific instance where there was clearly an indication that a virus, for instance, grew in a mish-mash, in a mixture of cells, that would otherwise have grown, nor the real reason why this should have happened. But the synergism between cells was certainly something that we attempted to develop. Kaposi's sarcoma cells were some early cell cultures that we were asked to grow. And I recently was on an airplane, on a flight to London--to Frankfurt--with Dr. Robert Gallo, who couldn't believe that there was such a thing as an early attempt to grow Kaposi's sarcoma cells. I believe that a number of these were subsequently sent to the American Type Culture Collection when our laboratory closed. But these were cells that he found of great interest because I believe that they had been trying to grow cells from Kaposi's sarcoma.

Stevenson: Back in the early days in the Virus Cancer Program, one of the initial

materials that were on a list that Harvey Scudder had elicited from the virologists as to what kinds of materials they would like to study, Hillary Koprowski had listed Kaposi's sarcoma as being one of the most likely cancers caused by a virus because of its clinical history and so forth, and he asked me early on, with the formation of the Tissue Procurement Program, could I obtain some for him.

Nelson-Rees: Interesting. But it wasn't until sometime in the late '60s or early '70s that we began obtaining these cells.

Stevenson: I couldn't find any back in the early '60s. They just hardly ever were observed.

Nelson-Rees: Interesting. And then there were specific tumor cells that Dr. Huebner was interested in. I mean, I, for instance, recall the time that the young son of--I believe it was--Edward Kennedy, whose leg was amputated because of osteosarcoma, and it was a really very furtive attempt to grow these cells in culture and, in fact, we did succeed with hurried calls to various agencies to get the tumor cells from the young boy to our laboratory and, I suppose, perhaps also for a political plum. But I'm merely mentioning that because it's indicative of the forcefulness and the nature of this particular program which, almost overnight, meant new tissues, new types of tissues, for a priority in culture because individual laboratories wanted specific cells to be grown and to be utilized and to be available which were thoroughly characterized and were guaranteed to be what they were said to be.

Stevenson: Incidentally, the surgeon who took the boy's leg off was my former boss, George Hyatt. He was the Head of the Navy Tissue Bank.

Nelson-Rees: How interesting. Yes. I'll never forget the day I sat there looking at the very first culture that had been available to us. Of course, when you

grow cells you have to make sure that you have a large number stored before you start utilizing them and using them up. So, the very first time that we had a chance to look at the young cells from the nephew of the former President, of the late President, I was astonished to be looking at the Y chromosome in a fluorescent preparation and realizing that that specific chromosome was not only that one, that came from his father, but the exact same Y chromosome that was carried by the late President John F. Kennedy. And somehow, or other, I thought that was an historical, if not hysterical, moment in my observations of cells in culture. But it was the same sort of thrill to find that as was the reverse, to find Y chromosomes in purported mammary tumor cells. It was an astonishing thing to find that just simply the presence, or the lack, of a Y chromosome could indicate contamination, when one was able to identify the Y chromosome. Of course, there are male human cell lines that don't carry a Y chromosome, but that is one of the many characteristics that we were able to utilize.

Stevenson: One of the questions that always bugged me a little bit, and I've never discussed in any great depth with you, is that with the understanding the HeLa cells contaminated so many of these early long-term cultures and later Dr. Stan Gartler of Seattle showed, with the use of isoenzymes, that there was a major contamination problem amongst the human cell lines, a lot of these had been thoroughly examined--the chromosomes had been reviewed and evaluated--on the passages of cells that had been produced for the bank. Some of them, like I remember the Minnesota EE line, or whatever, was supposed to have been a nasopharyngeal carcinoma from a Caucasian male, and yet the thing had been later found to be a HeLa displacement and so forth, but there was never any indication in any of

the certification process that I recall that pointed out that there was a lack of a Y chromosome in this particular culture. Was it because the techniques for chromosome analysis at that time were less precise and we didn't have Giemsa banding, that that wasn't detected?

Nelson-Rees: Well, Giemsa banding undoubtedly, and we took great advantage of Giemsa banding early on precisely for that very reason. This banding was based on R. Miller's and U. Franke's earlier descriptions of HeLa marker peculiarities. It is not always--Well, what you're saying is absolutely right. There was not always an attentive, trained cytogeneticist or chromosome person studying cells that were being utilized. But, more important is the fact that time and again it was clear that cells were assumed to be of a certain origin or of a certain specificity and were not tested before they were utilized in a major program in research. And not only that but, in a good many instances, while certain laboratories--Say, for instance, a laboratory that was going to study lung cells for a specific cause, for a specific reason, attempted not only to utilize lung cells which might have been available, but was also attempting in the new laboratory to initiate new cultures, and within these laboratories there occurred contaminations, intercellular cross-contamination, that had nothing to do with the original cells that might have been sent to them. So, contamination problems occurred not only in major laboratories, but also new laboratories, that were trying to grow individual cells for particular programs within these individual laboratories, and while the HeLa cell contamination is something which made great news, contamination had occurred much earlier, as you've indicated, between mouse cells, between rat cells, in laboratories of--

Stevenson: Syverton was one.

Nelson-Rees: Syverton was one of them.The big, husky man at NIH. Oh, I can't think of—

Stevenson: Earle?

Nelson-Rees: Yes. Wilton Earle's laboratory was already concerned. Katherine Sanford. They had had mouse cell contaminations early on, and they had detected them, and they were able to identify them on the basis of chromosome analysis, not only, but also other specific tests that they were able to apply, showing that there had been cross-contamination. But I think the Giemsa banding, and subsequently the C-banding fluorescent microscopy, Casperson's, fluorescent staining, much more clearly, much more readily, and much faster, was able to display contamination (iso-enzyme analysis). But Stan Gartler's epochal discovery really revolutionized, in more ways than one, the field. There was no question about that.

Stevenson: I think the thing that comes back to me in reviewing that historically was you get answers in terms of the questions that you ask. And we had not been asking questions as to whether we had intraspecies, or human-human contamination. We assumed that everyone who was taking care of their cultures were keeping them clean. We were concerned about interspecies contamination, so all of the program had been set up to develop reagents that would detect mouse, rat, dogs, whatever, different kinds of things, with fluorescent antibodies to determine that we had human cell lines. And then the sort of thing that would nail the whole issue down, was this a human cell line or not, was testing it with poliovirus to see that it would support the growth of poliovirus, because we knew, if it was a non-human cell line, it would not support poliovirus growth. So, at least with the tools that we had in our armamentarium, we

were doing a job of defining what we had, but we were unaware, or unable, to solve--or discern--the other problem because we weren't asking that question at the time. And that's why I asked about the chromosomes because, if we had been doing very sensitive and very penetrating chromosome analysis, we would have found that some of these human cell lines that were supposedly of male origin were, in fact, not exhibiting a key chromosome identification marker that should have been there that wasn't.

Nelson-Rees: Well, that's true. But we have to remember that the earliest cells grown, if I recall, were aberrant cells to start out with.

Stevenson: Yes.

Nelson-Rees: And when one talks about WI-38, or its precursor, WI-21, earlier cells of human origin that began to be grown primarily at the Wistar Institute, but also in George Guy's laboratory, endless attempts to grow cells, when you did finally get HeLa cells to grow, it seems to me that people lost interest in doing anything other than growing them. And they are marvelous cells. They're wonderful cells.

Stevenson: And they grow anywhere.

Nelson-Rees: And they grow anywhere. And so there may have not been any need to do anything other than grow them. And I frankly think that the people who are at fault, more than any others, in the early development stages of cells utilized for research, who led to the problem of contamination, were virologists who simply didn't give a damn what kinds of cells they were growing, just so their viruses were being grown. And the cells they were using, they didn't care what they were. They were growing their viruses and, from there on, they didn't particularly care to characterize cells.

Stevenson: Just a bag of enzymes.

Nelson-Rees: Yes. Just a bag of enzymes, exactly.

Stevenson: Well, this is often the case, where one discipline utilizes the fruits of another almost contemptuously without regard for all the lore and all of the problems and the associated specifications that are extant in the contributing field. I was straddling these two fields during that period of time and I was acutely aware of this not only ignorance of the problems of cell culture on the part of the virologists, but almost the contempt of, you know, why worry about all these things? Wilton Earle's silicone stoppers and all the elaborate glassware cleaning procedures; what difference does it make? You know? Why should we be bothered with this sort of stuff? The stuff grows our viruses. Leave it alone. Don't bother me with all of this stuff. And it caused a lot of mischief.

Nelson-Rees: You know, Bob, it's the sort of thing--I'm an avid listener to the O.J. Simpson Trial proceedings, and I just cannot understand--really, I just cannot understand--why every time the subject of DNA testing comes up, it's either the announcer, or either the defense, or the prosecution, or even the judge, alludes to the fact that it is *too complicated* to understand. We're going to-- on the 15th of December (1994), they're going to go into the Kelly-Frye Hearings to determine whether, in fact, the methods utilized in order to give results of the DNA testing are valid or invalid. And this is something which hounded early researchers in chromosome analysis, in immunofluorescence. Is the movement of the glucose-6-phosphate dehydrogenase Type A valid? Is Type B valid, as opposed to A? Human HLA testing, immunofluorescence all of these things, were early on questioned by a number of people. I remember Leonard Hayflick, for many, many, many years, absolutely refusing to accept the fact that the WISH--the Wistar Institute Susan Hayflick--cell line

[derived from his daughter] had been contaminated. It was never a purposeful attack on him, or the Institute, or his cells, or the carefulness with which the cells had been--but it was simply a matter of fact that it did exist. It had been contaminated. But people who were--

Stevenson: He still denies it.

Nelson-Rees: He probably still denies it.

Stevenson: He does. It's in his latest book.

Nelson-Rees: And so you're absolutely right. Why wasn't there more care taken early on to prove, or why weren't available methods-- even if methods were available, they were simply distrusted. They were ignored, or distrusted, or set aside, or given very, very, very low priority. And the human ego, the human mind, works in many different ways. If you are on contract and you suddenly find that somebody is finding that what you've been doing for 5 years, 3 years, on a certain cell line-- It not only hurts from the standpoint of ego; it's simply a whole matter of existence going down the drain. You don't tell somebody that he's been working with a HeLa cell that presumably has been seeking, searching and plodding along trying to define a lung antigen, when in fact it's the wrong cell line to be working with there. All sorts of factors enter into this that transcend whether or not it was accurately described and defined. The person who very, very honestly, and truthfully, and sincerely, and from the bottom of his heart came out and first acknowledged a great, great mistake was Bob Bassin in his HBT-3. I mean, he went out of his way to prove that his cells were, in fact, contaminated, and did so graciously and generously and--

Stevenson: And learned a lesson that he took to heart and never--

Nelson-Rees: And never repeated and never did again. But this was not the case with

many other workers, to this very day I would imagine. And so I'm sorry that the HeLa cell contamination has really taken precedence above everything else because, as you've alluded to, we had instances where we had multiple contaminations, where, once we had established Noah's Zoo and began to ship out cells to various individuals who would want to use marmoset cells, who would want to use rabbit cells, cat cells, dog cells, we had cell cultures returned to our laboratory where we found 2, 3, 4 different kinds of animals all existing happily together in one culture, none of which were the original putative cell. And so these were really very, very, very serious cases of contamination which never were necessarily talked about but, nevertheless, existed. Now I wanted to say one more thing--well, maybe a few more things. When you try to promulgate, or promote, the fact that a certain cell is contaminated, to some people, to some coworkers, to some colleagues, it seemed incredible that we would be specifying that it was one culture from one laboratory that had been contaminated. And the only way that we could assure readers of what was known then as the "hit list" could be sure that they were dealing with the contaminated cell is to specify the laboratory from which the individual cells came. And in order to do that we had to name the laboratory and possibly the person working with these same cells because, to have hidden that, to not have come out and said, "This was the [Stuart] Aaronson such and such cell line; this was the cell line that came from Elizabeth Priori," it would be futile because it was only that particular cell culture that was contaminated and, if another colleague had obtained the same cell for study, knowing that it had come from the fingered, or the guilty, laboratory in this particular case then all other cell cultures of that same family would be suspect.

Stevenson: It's interesting, your comments earlier about the O.J. Simpson thing. There is a parallel here. The problem with all of this type of forensic material is quite often not the end test itself but the chain of custody of the evidence as to where the evidence was picked up and who had their hands on it between the time it was collected and identified and it reached the laboratory, because things can get contaminated with other materials in the transit process, or samples can get mixed up if they're not properly labeled, or samples can be missing if someone has lifted samples that were taken from a crime scene and these disappear. All of these things go into it and, when you do this kind of "sleuthing," as I call it, it is critically important that the chain of custody be well documented, as well as the sensitivity of the final tests that were applied to the material.

Nelson-Rees: Yes. The sensitivity is something which is now very much in question. How reliable is the immunofluorescence test? How often can you expect, in a population of 100, to find the same movement? How often does it occur in a million? Can it be more than one, or is it only among identical twins that the same occurs? Of course, one of the things that doesn't come up today is that one is looking at only very minor, very few, characteristics. Very, very few characteristics will be utilized in the DNA testing because these are the only ones available at the moment with polymerase chain reaction (PCR) or Restriction Fragment Length Polymorphism (RFLP). But still, it's the lack of belief, the distrust, that is being utilized in this particular case by the defense, the distrust that any of it is any good is the same sort of thing that happened early on in the Bedford Spring meeting (Decennial of the ATCC) when people absolutely distrusted any kind of value--

Stevenson: --of the isoenzyme procedure?

Nelson-Rees: Exactly, to the enzyme procedure.

Stevenson: Well, you know, I served on a committee that was chaired by John Caskey for the Congressional Office of Technology Assessment. I forget its exact title. But that was at least three years ago. And a group of experts came in and brought in all the evidence for these probes and everything, and I think, to me, there was no question as to the scientific validity of the procedure. And one has to assume, of course, that the manipulations are aptly done and that the technicians are trained to do the procedures properly but, given that as a base, the results with these various probes have, you know, a fantastic ability to detect exact patterns of these DNA profiles. And to have the chances of these things simply by chance being replicated in a population are so high--one in tens of millions--that given a specific crime locus, if you do, in fact, match up a suspect's DNA with that that came from the crime scene, you can, without any doubt, say it came from this suspect.Now, the question as to what the significance is, is not illuminated by the scientific test. And that, taken in conjunction with the problems of chain of custody, you know, you can have perfectly logical explanation why someone's, some specific individual's blood, is in a particular place. But then the detective work and the other things--circumstantial evidence--has to show the significance of that. So, the science, by itself, can't prove or disprove.

Nelson-Rees: Yes. There's often talk about contamination. I think that if one assumes that there is contamination, one mustn't so glibly do away with the fact that you might be able to artificially contaminate. Assume that you have--go ahead and mix two different bloods. You have the people living, or you have samples, ample samples, which you could mix and see what the

mixture itself revealed. I doubt that soil, I doubt that amoebae, I doubt that spores would in any way contaminate in a manner that couldn't be identified as such. So, in the case of HeLa, it's also something else that I think we must remember, and that is that you cannot recall cell lines or cell cultures like you can recall trucks or automobiles of a certain year. Once cells are distributed worldwide and continue to grow, you will have, by genetic isolation itself, you will have evolutionary changes occurring in cells that have been separated from one another, and each other that simply might lead to differences in cell cultures. In other words, all HeLa cells are not alike. You will have subcultures of HeLa cells as you will have subcultures of species eventually developing through isolation into different cultures. And it's quite likely--and we did a paper one time--we did a study one time--of a variety of different HeLa cells from different laboratories, different parts of the world actually, and there are significant differences in chromosomes, there are significant differences in ability of cells, when inoculated into hamster cheek pouch, to produce tumors. There are differences in the actual marker chromosomes which were so important early on and which remain very important as markers of HeLa cells. So, one has to be very careful what it is-- There were even cells that did not grow poliovirus, or didn't exhibit CPE, that were bona fide HeLa cells that simply behave differently.

Stevenson: I remember there was an S1 variant.

Nelson-Rees: Exactly, exactly. Our primary contacts were Stulberg, and Coriell, Ward Peterson, Arthur Green, and I cannot think of the chromosome man at Camden, at Coriell's laboratory, a very fine, very good, good worker. I cannot think of his name. He did chromosome work. He and Levan

worked together. I referred to him earlier.

Stevenson: Wasn't it Warren Nichols?

Nelson-Rees: Warren Nichols. Thank you. Yes, excellent worker. A very, very careful worker. He did some beautiful work with moths versus insect (mosquito) cells--magnificent work--very early on, very good work.

Stevenson: I don't know what's happened to that bank now. The derivation of materials there went on at a great rate from genetic mutant cell lines. They were collecting large numbers of people with genetic abnormalities. Then Art Green retired, and I don't know who, now, is in charge of the cell bank there. One of the fellows who was at NIH and was the Project Officer in Kirschstein's institute for the Coriell contract left NIH, went to the New York Public Health Laboratories in Manhattan, then came back as Director of the Coriell Institute, a fellow by the name of David Beck. He had been at NIH and I knew him slightly. But he is not, per se, you know, involved in the cell culture activity, but I don't know what's happened to it. And I would think, with all of this business going on with the Venter and other DNA delineation projects that some of those things might be useful probes to match up with the bits of DNA that are being isolated to see if these do match up with any of these specific cell lines that came from families with genetic abnormalities.

Nelson-Rees: Well, Dr. T.C. Hsu and Dr. Margery Shaw are some people like that who were working a lot with genetic abnormalities and specific cell lines derived from them and their identification, not only from the chromosome standpoint, but from the biochemical approach. What is it that these cells do that other cells don't? Or what specific things delineate the biochemistry of this individual? These were things that were of very, very--and do they, or do they not, produce different

antigens, or antibodies in response? And those are a wonderful adjunct. But you know, Bob, as we're talking about these things, only very, very few laboratories worldwide are capable of doing these in-house. And whether or not these many, many research laboratories worldwide take advantage of the likes of ATCC for studies--

Stevenson: I can tell you they don't.

Nelson-Rees: They don't? And, you see, the last time you mentioned a conference at--I think it was--at Silver Spring that was led by Dr. Jack Gruber of NCI about 3, 4, or 5 years ago, that brought the events and research of the Virus Cancer Program up to date then, there was there a talk about the fact that many laboratories--many laboratories--were exhibiting contamination problems. And this was Ward Peterson telling me, Ward Peterson who was, at that time, still with Stulberg. He had an endless number of contaminations.

So, it seems as though, while many people know about it, history is repeating itself time and again. And I hate to always be associated--and I'm far removed from this now--but I hate to hear people say that the problem has been solved. It hasn't.

Stevenson: No. Each new generation of scientists has to relearn these basic problems and put measures into effect in their own laboratories to guard against them. One of the things that interested me was that over the years we supported from NCI's Virus Cancer Program first, and then later it was done at Frederick under the SVCP, a testing laboratory to detect *Mycoplasma* contamination in cultures. And, although the percentage in any laboratory of contamination had gone down, the fact that many new laboratories were coming on line, and so forth, the number of positive specimens that were being submitted to that testing laboratory

maintained the same proportion, even though the numbers of tests had gone up almost five- or six-fold. So it indicated to me that this was an abiding problem and that the only-- Like someone said about liberty, "The price of liberty is eternal vigilance," and the freedom from contamination is eternal vigilance. And if there is no ready and economically available means to do this testing, then you're going to come back to the same days we were in the late '50s when all the materials that are being traded around are contaminated. One thing that is a problem is that ATCC, for example, cannot maintain different categories of material and put out a cheap cell line as a standard reference culture without Government subsidy. The cost of the production and so forth is such that you have to charge a reasonable price to send a laboratory a culture. They don't buy them in bulk and they don't set aside standards by purchasing in large lots these cells to reduce the unit cost of them. And so, occasionally, laboratories that are pretty sophisticated just simply send for a new culture and start their stocks from freshly obtained cultures and tend to chop off any continuing contamination problems by starting with new seed. This is an old technique that farmers have used for centuries. But the point is that unless, and until, there is some kind of a useful, economical testing service set up where these lines can be sent in and re-evaluated, this is going to be a continuing problem.

Nelson-Rees: Are cell cultures used as much today as they were 20 years ago, 10 years ago?

Stevenson: Well, certainly from the standpoint of distribution at the American Type Culture Collection. They have been consistently growing in distribution every year when I was there for 13 years, and substantially. I mean, it

was like a 10 percent--

Nelson-Rees: Every year?

Stevenson: Every year. So that the amount of cultures now that are going out are quite substantial. But also you were seeing a lot of new cultures that come in. Now, you have like books on the bestseller list. You will get in new cultures and, for a period of time, these may--

Nelson-Rees: They're fashionable?

Stevenson: --they're fashionable and they distribute more. But then you have the old stand-bys, the WI-38's, the MRC-5's, the HeLa's and so forth, and these tend to, you know, just have a very steady distribution over months and years and continue to be widely utilized by all kinds of research people. So, it's an absolutely vital thing to maintain, but the problem with getting new ones in and ones that aren't patented, or ones that are patented and people put restrictions on them and so forth, it makes life a little dicey at times for the bank managers but it's, nonetheless, still useful.

Nelson-Rees: Are there any new areas of research that the cells are being used for that you think are absolutely novel areas for research with cells?

Stevenson: A couple of years ago a brain cell was deposited, and that is patented, but I have a feeling with the interest in Alzheimer's and so forth, that there will be a whole category of brain cells now that will be developed and widely utilized.

Nelson-Rees: What about cells that have been discovered that exist in homosexual males and not in the general population? Granted, cells and work done on heterosexual males that have died, there are a number of those.

Stevenson: Are you thinking about the brain research, where someone has detected a putative difference?

Nelson-Rees: Brain research. Yes.

Stevenson: I don't know that anyone has reliably cultured brain cells of that type to an extent that you would know that you had specific material from putative cases. So, it's an interesting possibility. I think all of these things that one wants to study physiological mechanisms in will result, at some point, in people trying to tease out the growth factors and the culture conditions that enable one to propagate the cells. But the brain cells, particularly neuronal cells, are extremely dicey in terms of how to do them and everything but, you know, this will yield in time.

Nelson-Rees: Are there monies for research now, still, granted for the likes of characterization laboratories?

Stevenson: I really don't know.

Nelson-Rees: You mentioned a Japanese group that is trying to--

Stevenson: Well, these were paid by WHO to grow a batch of cells.

Nelson-Rees: I see.

Stevenson: There was a fellow in France who created the BHK cultures at the Pasteur Institute, and those were supposed to be split up and sent to repositories at ATCC and Japan, and then some kept in France. But, if I recall, when he shipped the material to the United States, it thawed in transit, and stuff, and we got material that had been in the pipeline quite long and the cells, a substantial portion of the cells, had thawed out so that they were not transported under ideal conditions. They were tested and found to be viable and were refrozen but, you know, whether this would have any effect-- I would be very leery about using these as an international standard because of that kind of a mishap. But, again, I suppose if one tests them and finds that they're okay, it would be-- It would be a job to do it.

Nelson-Rees: You know, having been away from this for a while, I still wonder

whether people are--We figured, with lesser connotation, cells that were presumably lung cells that turned out to be not lung cells, breast cells, liver cells--I'm talking about specific instances--kidney cells that were not kidney cells. I wonder, have these programs ceased to exist? Has one finally got the right lung cells, the right kidney cells, the right breast cells, the right prostate cells, and by "right" I mean bona fide cells, and have these programs changed? Have the answers been found and has one gone on to other things?

Stevenson: I don't think anyone has really directed sufficient attention to what they expect out of an *in vitro* system in many cases.

Nelson-Rees: That's really what I'm asking.

Stevenson: People go to the catalog and a cell that was derived from a particular tissue they seem to think is endowed with all of the attributes of that tissue, or the organ from which the tissue came, a liver for example. And yet, even a casual acquaintance with the physiology of the body and the tissues and the organs from which these things are derived, would tell you that these things are very, very complex systems and that to look upon a 2-dimensional sheeted-out culture as being representative of the 3-dimensional organ that has all kinds of different connections with other types of tissues and cells and so forth, as being representative of what goes on in the body, is absolutely beyond the pale of imagination.

So, I think a lot of things are done very naively from that standpoint; as naive as people are in thinking that everything that they get that has a label on it must, in fact, conform to what the label says.

Nelson-Rees: Yes. We had a terrible time determining whether, in fact, when we grew breast cells, or lung cells, or kidney cells, we were not actually, in fact, growing connective tissue.

Stevenson: Right, and the chances are you were.

Nelson-Rees: And the chances were--not only the chances--but, in many instances, that's all we were growing. And, as frustrating as it may have been, this was the case. So, I felt that very early on Chang liver cells were not really liver cells that one sought for their activity as liver cells, any more than lung cells, any more than, of course, breast cells, and so, one hopes that one has the handle on good cells today that really represent those particular ones. I can't imagine that baby hamster kidney cells, or Viro cells, or HeLa cells, even when set up as standards, will satisfy--give answers--to all of these particular--

Stevenson: No. They won't. I think the reason that those were set up is that at least for viral diagnosis for viral vaccine substrates some of these things are thought to be extremely useful and, therefore, since they are used so widely in so many different places, either for diagnosis and/or vaccine development, that they should be standardized. And I strongly support that. But that doesn't mean, by extension then, that these things are useful for everything; it just means that they have a very specific, highly useful connotation for certain applications, and that's it.

Nelson-Rees: What about HIV? Does it grow readily in human cells and, if so, which?

Stevenson: Well, here again, I think that is a very complicated thing, having to do with different strains. Some strains seem to grow quite well in tissue culture; others don't. I think you can't talk any more about "a" or "an" HIV strain; you have to specify which strain you're talking about and its cultural history, because some are attenuated, some are, you know, virtually useless. They found this out recently with trying to produce some vaccines, that these things bore very little relationship to the current strains that are actively infecting people in different parts of the

world. Strains, for example, in Africa, are much different from strains found in the United States and Asia. So a universally useful vaccine made from a strain of HIV is probably already a physical impossibility. You would have to have multiple vaccines that are developed. This is one of the things--You know, we still have a large number of cases of trypanosomiasis (T. gambiense) in different parts of the world, and these organisms have the uncanny ability to almost instantly mutate when they are faced with an antibody.

Nelson-Rees: *Trypanosoma* itself mutates?

Stevenson: Yes.

Nelson-Rees: How interesting.

Stevenson: And it's like you're trying to catch a thief in a department store and you grab a hold of the coat and the thief just simply goes out of the coat, escapes bodily, and so forth, and you're left with the coat in your hands and no thief. Well, this is what has happened with immunization against trypanosomiasis. You create a vaccine that, in essence, grabs the coat, but the organism escapes and goes on then to put on a different colored coat of different material and the vaccine that you had initially has no effect whatsoever on this new coat that the organism develops. It would appear that the AIDS virus, you know, does something similar and mutates very rapidly so that it's almost-- if you remember mythology, there was a man named Proteus, and whenever he was grabbed he would turn into all kinds of different animals.

Nelson-Rees: This is why you call it *Amoeba proteus*.

Stevenson: Yes.

Nelson-Rees: Not to mention *Trypanosoma gambiense*.

Stevenson: Right.

Nelson-Rees: Why did our laboratory cease being funded? Do you have any idea?

Stevenson: I assume that Huebner had a limitation of money and just simply moved on to things that were of more immediate interest at the time. I had long since been away from NIH, so I'm not privy to any of the policy decisions that shut it down.

Nelson-Rees: But I imagine that their emphasis has, as you say, in general, has switched to other things.

Stevenson: But that was often the case with a lot of those programs. When money got tight, they reprioritized what they were doing and moved on.

Nelson-Rees: I must say that all the time from '61 until '81, twenty years, we had the very, very best support, very best support. We had to fight for it on an annual basis. Not fight--we had to defend the program and we had to reapply for a renewal of a contract--but we had the facilities to do clean cell culture and to have, not on a grand scale-- As Dr. Huebner used to say, "We cannot afford to have an institute at every contract facility. It can't be an institute; it has to be a limited laboratory with a certain scope." But it was extraordinary the extent to which we were supported, but I must take some credit for having had a great interest in maintaining what we had and promoting it and fighting for it. I used to get very upset when a worker would come and say, "Well, Dr. Rees, this handle has been gone for a month." "What do you mean?" "The door handle to the storeroom has been gone for a month." "What do you mean by that? Why wasn't it repaired, changed, maintained?" And this sort of petty housekeeping is something that apparently led to something worthwhile. It's something that I could do, and I enjoyed doing.

Stevenson: When you think back and reflect upon those 20 years, what would you enumerate as the significant accomplishments that you feel good about

that the laboratory did?

Nelson-Rees: I think that we initiated many difficult cell cultures and that we were able to produce larger quantities of them. We were able to coordinate, collaborate, work with other people, to grow specific cells for them and for our own in-house research. And I think to have had lists, ultimately to have produced a large variety of cell cultures that suited other peoples' research programs, was a very rewarding thing. And then, when it came time to make the "hit lists"--It was good to hear a man at Fordham University (I believe it was Dr. Fritz Herz) once say that before he ever had students work with cell cultures, that he sent them to see the lists that had been published in *Science* magazine that indicated, if not that they were contaminated, but that list, that cells with that name might be contaminated and should be checked before they--So, references to good, clean cell culture, ways of culturing cells, and cultures that ultimately were of value. And we did a lot more than just put a finger on cell contamination. There was a large number of other cells that were valuable, that were important to people, and that served the purpose. And I thought that these were satisfactory.

Stevenson: Could you distill, from these experiences, any general precepts or generalizations that you feel would be useful to state as such in terms of the philosophy or the practice of cell culture, or experimental manipulation, or general advice? In other words, what did you learn that were abiding principles, or lasting injunctions, from this type of activity?

Nelson-Rees: By nature, I think that I'm conservative. By nature, I think that I withstand change. By nature I think that what I can contribute to a cause is continuity. I don't ever seek something brand new. I don't necessarily want to change the course of events. This may be *retardataire* in a

certain sense. It may want to prevent evolution. But I think once one has established certain patterns, that one should stick with them.

What I'm saying, Bob, is that towards the end of the existence of our laboratory, the people who were doing research in the laboratory were trying to do too much. They were trying to apply for new contracts to do many different things other than clean cell culture initiation, cell cultivation, cell preservation and distribution of known product. It was something new that had to be done, something new that wanted to be applied to getting a new contract to do something else. We had gone from a strictly characterization program to--

Stevenson: Novelty.

Nelson-Rees: --to novelty. I suppose that's the best word. I don't think that success lies with novelty. The hula-hoop syndrome, the skateboard, the something new all the time, it does not always have to be a new type of banding program, a new type of staining, a new type of-- I don't know whether this is good or not, but it satisfies me as an individual to know that what I do on a very limited scale is done right all the time for ever after. And this is the way I have functioned and this is what I was good at, at the time. Whether it would still hold, or not, I don't know. I used to sit in conferences, in symposia, and other meetings and I used to marvel at what people were saying and what people were doing, and I thought that success was around the corner, and I thought that we were getting solutions to a number of problems. And when I'd think on it, and when I go back now, many years later, I find that many of these things were hot air. They were, to some extent, scams. And maybe it's because old people tend to be skeptical and tend to be in a rut. I don't know that all of that is true, but that's how I felt for many years.

Stevenson: Do you feel that there is a lack of general support, or that there is a need for some kind of abiding standards institution for this type of material; that it is in the best and most economic interests of the government to see that some kind of authentication and validation capability is available?

Nelson-Rees: Absolutely if, by "Government," you mean something that is non-politically oriented. It's by something that has to maintain, year-in and year-out, in a working, solid condition that is forever a basis for-- What should I say? It has to maintain forever without being changed because of a political change. It just simply cannot exist without permanence.

Stevenson: One of the things I was impressed with, several years ago I took a trip to Turkey and went into the museum in Constantinople, and here were standards of length and volume which had been established by Hammurabi. I mean, it went back, what, 1750 B.C. And those had been used back in those days so that traders had some kind of a reference and standard by which they could do commerce. And this was recognized early on in the very earliest civilizations that we've had.

Nelson-Rees: The equivalent in Western culture is the CGS System. The *Academie Francaise* in Paris has the centimeter, the gram, and the second standards.

Stevenson: Right. But we don't have this in biology. In fact, some people think it's paradoxical to think that one could have standards which apply to living systems. But, on the other hand, when you don't have some kind of international currency which is recognized by everyone as being valid and a benchmark by which things can be tested, then you run into chaos and, as the chaos increases, then you become frustrated and you waste a lot of money and effort in doing things because you don't have the standards. So, I think the idea of the standards is a very critical one, but I

don't know exactly, since these things are not amenable to the same kind of legislation that the length of the meter and the strength of the volt and so forth are, how one comes up with that. I think this concept of having batches of similar cells available to people at quite reasonable or no cost through this WHO Program is probably a very good beginning. I'd like to see more of it frankly. But again, you have to have the ability, once you get this material, to either go back and get fresh seed, or to be able to test what you are manipulating from time to time to make sure that it has not deviated from the base from which you started. That's wherein comes so much of the problem is that people assume that no untoward things happen; that you carry a culture uncontaminated for years, whereas, in fact, every time you open the stopper on the bottle, the chances of something getting in are finite. It does occur and will occur and there is no way around it, as long as you're using these materials and transferring them.

Nelson-Rees: Of course, I take back something, and that is it would be nice if you had a universal antibiotic or a universal cleaner-upper for cell cultures. If you could clean up a cell culture that had been contaminated, say with microorganisms, or *Mycoplasma*, or PPLO, it would be good to have developed something that was a broad spectrum cleanser which would allow smaller, less fortunate laboratories to work under less stringent conditions and still work with good cell cultures. I don't know whether irradiation, which obviously is not--irradiation of media, of dry media, of powdered media--whether these things would not change molecularly, whether the structure doesn't change but, at any rate, whether or not it would be possible to develop. I remember you saying one time, however, Bob--and it was a long time ago, I think, at Lake Placid--you said that,

"We worry so much about the purity of water that we're utilizing in cell culture, all the while there is no such thing as pure water that we ever ingest, or drink, or have about us, or swim in, or wash in, or cook with but, for the benefit of the cell, you must have certain kinds of good water, water with a certain standard." But it'll never be pure. It'll never be absolutely--

Stevenson: Water is the universal solvent.

Nelson-Rees: That's right. That's right. But it would be nice. There are lots of things one could think of to make it easier to grow good cells and so, from that standpoint, I admit that they must not always be the same prevailing-- But I fought many, many times with having to watch when someone--or having to combat somebody's--wanting to change what was well established. It just was against me. And, if I succeeded in maintaining that, I'm happy.

Stevenson: Apropos of that then, what do you think about Kuhn's idea of scientific revolutions?

Nelson-Rees: Well--

Stevenson: Are you one of the "holders back," or whatever, in that you don't like to see the wave of things developing?

Nelson-Rees: I don't think that in the next 15 or 20 years which I have, I think, left me, I need to have anything new. I really don't think that I need to have anything new happen.

Stevenson: But it will.

Nelson-Rees: It will, yes. But, you know, you go to Paris and you walk across the *Place de la Concorde*, and it's always the same *Place de la Concorde*, or the Queen's Garden, or the Eiffel-- It's always the same. You go to downtown San Francisco, and that God-damned park in front of City

Hall here, every time you turn around they're planting a new tree or they're putting in a new fountain. And it's novel, and it's novel, and it has to be changed, and it has to-- And that simply does not have to be, in my opinion. It can always be a majestic, open, plaza--a square--and it doesn't always have to change in order to be grand and beautiful and desirable and everlasting. And this is idealized talk because, you know, I just think things in life don't always have to change. I'm sure you're right--they will and they have to.

Stevenson: Well, it's the old French saying, "The more things change, the more they stay the same."

Nelson-Rees: Stay the same.

Stevenson: How do you interpret that in view of what you just said?

Nelson-Rees: Well, history repeats itself. Whatever. But personally I don't think it's-- It would be not necessary to develop a new system of growing cells.

Stevenson: Supposing, all of a sudden, I could present you with a technology that you could grow cells in 3 dimensions in mixed culture? Would you find this interesting and stimulating if you wanted to look into organogenesis and the way in which you could play with, or develop, miniature organs *in vitro*?

Nelson-Rees: Oh, I don't think that that-- Well, that would be--- Yes. I see what you mean. But that's a different category now. That's applying good cells to a different system. Oh, that would be very interesting. Oh, that would be extraordinary.

Stevenson: I don't think you're quite as hide-bound or conservative as you might think that you are. I think what you're saying is that you want to see a certain continuity of useful applied things maintained and perpetuated so that you can have repeated assurances that what you're dealing with is, in

fact, authentic material. But I think you would be the first to seize upon and utilize any improvements that came about in the technology by which you could assure yourself of that continuity of purpose and propagation. Isn't that correct?

Nelson-Rees: Probably so. Yes. I think that's--I'll acquiesce. I'll grant that.

Stevenson: What we're arguing is the perpetuation of virginity versus constant loss of it. In other words, once you've maintained something, or established something, as a pure, uncontaminated entity, you're perfectly content to see that be perpetuated as such, and that the evaluation of how that is done should not be subject to constant whim and change and uncertainty but, rather, that certain well-established principles should be maintained.

Nelson-Rees: Another thing I think--

Stevenson: Quarter to five.

Nelson-Rees: One of the things that I think about a lot is writing by longhand and using the dictionary and using a grammar book versus having a computer do it necessarily for me and using the dictionary in the computer. Lately I've been involved with a lot of proofreading and content reading of art-related matter, and it's astonishing how much the computer does not know that you would have known had you troubled yourself. So, solely relying on a new method of writing a book, that is sitting in front with a Mac and a mouse and writing away and thinking that the computer itself will organize and place your sentences in the right place, even with cut and paste, even with the use of the dictionary, the computer, if you don't read the final galley and correct it, it will never be a good presentation because it's flawed beyond belief. And that use of something magnificent as a computer, it will not do unless you know how to spell, unless you know punctuation, unless you know what "ellipses" are,

unless you know where apostrophes go, and don't go--ever, ever, ever go--it will not happen. And that's perhaps the sort of thing I'm talking about. Oh, God, I think computers are fantastic but, unless you, the individual, don't know how to do the cell culturing the way it's done properly and depend on something new that comes up, it just won't work. You'll be back to the old days.

Stevenson: As someone once crudely put it to me, computers are dumber than whale shit and they are only tools that you can use.

Nelson-Rees: They're wonderful tools.

Stevenson: Yes, but you can cut yourself with them, or they can be blunt as hell.

Nelson-Rees: They're just terrible. They're just terrible. Just unbelievable. I think I'd better go and meet my friend, but do come with me and meet him.

Conclusion of Interview