

Harry Burruss Interview

This is an interview with Mr. Harry Burruss at his home in Gaithersburg, Maryland, April 17, 1986, about his work preparing yellow fever vaccine during World War II. The interviewer is Dr. Victoria A. Harden, NIH Historian.

Harden: Mr. Burruss, would you talk a bit about your training and how you came to work with the Rockefeller Foundation?

Burruss: I completed a three year, pre-medical course at Harvard College and planned to go on to medical school. Unfortunately, I did not have the funds necessary, so I started to work before going on with my studies. A prep school friend of mine in Cambridge, Massachusetts, knew a person who had just left the Rockefeller Yellow Fever Commission in Lagos, Nigeria. He had worked as a technician. He said that the director of the Rockefeller Foundation was looking for a replacement for this man, and that he was coming to Harvard Medical School hoping to interview someone. I jumped at the chance to interview with Dr. Frederick F. Russell, then the Director of the Rockefeller Foundation. After several months, he offered me the job in Lagos, Nigeria, as a technician with the Yellow Fever Commission.

Harden: What year was that?

Burruss: That was in 1928, in the fall of 1928. The Rockefeller Foundation had four or five doctors doing research work on yellow fever. At the Rockefeller Foundation in New York, research on yellow fever was being conducted with the development of a vaccine constantly in mind. I stayed with the Rockefeller Foundation West

Africa Yellow Fever Commission in Nigeria from 1928 until 1933. At that time they were considering closing up the work in Lagos, so I returned home and went to work for the Rockefeller Foundation as a technician in the yellow fever research work in New York.

Harden: What kind of work did you do in Nigeria?

Burruss: The doctors there were testing the behavior of yellow fever virus in monkeys to see if they could develop some sort of vaccine. Also, Dr. Wray Lloyd was trying to attenuate a virulent strain of yellow fever virus by passing it through mouse testicles. He worked on that for, I imagine, the best part of two years. Then there were several doctors working in the field trying to find active cases of yellow fever. They would send in samples of the victim's serum to find out if it was immune to yellow fever. They did this by using the so-called yellow fever protection test. It was done by injecting mice with a mixture of French neurotropic yellow fever virus and human serum. The results told them whether the person had had yellow fever or not. They were doing surveys in the field to test what percentage of the people showed immunity to yellow fever. There was also an entomologist, Dr. C. B. Philip, working with mosquitoes. He was trying to find out what species of mosquitoes would transmit yellow fever. That was an important part of the work. They would feed different mosquitoes on monkeys to see if those species of mosquitoes would transmit the disease. In 1933, after working for several months in the New York laboratory as a technician, I agreed to go to Brazil to work as a technician in the yellow fever laboratory the Rockefeller Foundation had set up there. They were doing the same

type of work in Brazil that they were doing in Nigeria: serological surveys in different parts of Brazil to find out where yellow fever was endemic and entomological studies on what species of mosquitoes would transmit yellow fever. My first station was at the laboratory in Bahia, Brazil. My principle job was testing blood sera to see what percentage of the population in a given area was immune to yellow fever. In 1936 the Bahia laboratory was closed, and I was transferred to the main laboratory in Rio de Janeiro.

Harden: Why did they close the lab?

Burruss: They wanted everybody in one place. The Rockefeller Foundation originally had facilities in Brazilian government quarters until they built a new laboratory with more space. They thought it would be more efficient if everyone worked in one place. I continued this work of testing blood samples from all over Brazil to find out where yellow fever was endemic. At the same time I was able to do some little research work on a young-mouse neutralization test. The test that we had been using was done with adult mice. I found out, with the help of Dr. Loring Whitman, that the young-mouse protection test was a little more sensitive than the one with adult mice.

In the meantime, of course, yellow fever research work was also carried on in New York. Dr. Max Theiler was one of the principal men there, but there were four or five other doctors working with him. And they developed the 17D-strain of yellow fever virus, which is the basis for the yellow fever vaccine now in use. The 17D-strain was developed from the Asibi-strain of yellow fever virus,

which had been obtained from an African native by that name. It was maintained in a culture containing embryonic mouse tissues for eighteen subcultures. After that, it was maintained for fifty-eight subcultures in a medium containing minced whole chick embryos. Then the medium was changed to chick embryo tissue, without the brain and spinal cord. It was only after 160 subcultures the 17D-strain was evolved.

Harden: As I understand it, no one really knows what caused the virus to attenuate, because other attempts had been unsuccessful.

Burruss: Yes, even Dr. Theiler said he didn't know just why. But apparently somewhere between the 90th and 110th passage something happened to attenuate the virus. And, of course, that is what they wanted.

Harden: I think someone said that, for all anyone knew, it could be attributed to a tugboat whistle blowing in the East River at just the precise moment!

Burruss: Once the virus had attenuated, the research workers in New York, started making vaccine from this 17D strain. They set up a yellow fever vaccine production laboratory. In 1937 they sent a quantity of this 17D strain to Rio de Janeiro with Dr. Hugh Smith. It was used on a large number of people in clinical trials to verify that it was as safe as they believed. All of this work took years to accomplish. The people in New York were working on a vaccine in the late 'twenties, but it wasn't until 1936 or so that the vaccine was finally developed. I continued with the laboratory work until about 1938 or '39. Principally I did neutralization tests and some research work on a new neutralization test or

“protection test” as some people call it. My last year in the Rio lab was as an administrative assistant. In 1940, I left Brazil. There was talk of bringing the yellow fever work in Brazil to a close in the near future. By that time I was ready to come back to America for vacation, and I decided not to return to Brazil, because they were going to close the laboratory there soon.

In the meantime—I don’t remember exactly what year—Dr. Mason Hargett was in Brazil. He had come to study the technique of yellow fever vaccine manufacture and also to learn about the research work they were doing in the field. As I recall, he spent about a year there. As you know, he was eventually given the job of manufacturing yellow fever vaccine for the Public Health Service. In 1939 or so, the Rockefeller Foundation wanted to stop producing yellow fever vaccine. They felt that their research work was finished, and they wanted the Public Health Service to take over the manufacture of yellow fever vaccine and, of course, that’s how Dr. Hargett got involved. I had returned to the United States in the fall of for several months’ vacation. I wasn’t sure what I should do next. The Director of the Rockefeller Foundation offered me a job in the New York laboratory. While I was thinking it over, Dr. Hargett got in touch with me and told me he would like very much to have me come and work with him in the new yellow fever vaccine lab that he was going to set up at the Rocky Mountain Laboratory in Hamilton, Montana. And after thinking it over for a while, I decided that I preferred to work in Hamilton with him. That’s how I got started in Hamilton.

Harden: Dr. Hargett said that you and he had the opportunity of creating the new vaccine unit exactly as you wanted it because yellow fever vaccine production had never been done in the Public Health Service before. I presume you got the seed virus or the vaccine from the Rockefeller Foundation.

Burruss: Yes, the 229th passage of 17D-strain of yellow fever was received at Rocky Mountain Lab from the Rockefeller Foundation as Lot 145-3 in January 1941. Columbia No. 88 was the seed virus used to prepare Lot 145-3. The origin of Columbia No. 88 is given by Sawyer in one of his publications. At the Rocky Mountain Laboratory the following passages of chick embryo were made: Lot 145-3, from the Rockefeller Foundation, was injected into egg embryos and we called that Lot YF1. Lot YF1 was passed to another, Lot YF10. And YF10 was passed to Lot AB237, which was set aside as a primary seed virus. Some of that lot was passed to AB616 and called our secondary seed virus.

Now at this time, it was decided to that we should stabilize the passages to prevent any changes that might take place by continually passing from lot to lot to lot such as the change that took place when it was developed. We certainly didn't want some bad change to take place. That is why we set aside Lot AB237, which we knew was a good titre. It was not used for anything except to make a new lot of secondary seed virus. Now let's say we had 400 ampules of the primary seed virus. We could take one ampule of primary seed virus AB237 and make one lot of secondary seed virus containing possibly 1,000 ampules. The primary seed virus would last for many years using that system.

Harden: How did you store the primary seed virus?

Burruss: It was desiccated material stored under dry ice refrigeration. Under those conditions, the primary seed virus would keep for years. The secondary seed virus was stored as a frozen product under dry ice at about -78°C.

Now, I know you wanted to know about setting up the lab. It was a very slow process. We had the advantage of seeing the setup in Brazil. We attempted to duplicate what we saw there and improve things that seemed to need improvement. One of the first things we did was to have a sterility room built. It was equipped with ultra-violet lights to make it as sterile as possible. And then we had to set up our animal testing quarters in an isolated section apart from the vaccine production. During the war, when we needed as much vaccine as we could produce, we were on a six day working basis. Actually, it was a seven day working basis, but on Sundays we timed it so that we didn't have too much to do. The process we followed was to inoculate eggs, to harvest a batch of eggs every day, to desiccate a batch of the liquid vaccine every day, and to test it in animals everyday. It was a continuous process when we were in full production.

Harden: What kind of animals did you use for testing?

Burruss: Principally, we used white Swiss mice, guinea pigs, and rhesus monkeys. Now, I want to go through the entire process beginning with egg inoculation. Maybe I should show you some of the pictures in this notebook I prepared.

Harden: That would be most interesting.

Burruss: As I said, to make yellow fever vaccine, everything had to be bacteriologically sterile. All of our equipment was sterilized. This first picture shows the cabinets containing the sterile supplies we used in production. We bought fertile hens' eggs and put them in an incubator for seven days. The incubator had an automatic turning device that every four hours would turn them about 45° back and forth. That was considered good technique for hatching chickens—our incubators were regular chicken-hatching incubators. After seven days, we would candle the eggs and throw out all the eggs containing dead embryos. At that point the good eggs would be inoculated with the yellow fever seed virus. The seed virus had previously been titrated in mice to see how potent it was. We would adjust the potency of the seed virus to about 40,000 LD50 per ml. "LD50," of course, means fifty-percent of a lethal dose for the experimental animal being used. As we prepared to inject the eggs, we diluted the seed virus with saline so that it contained 2,000 LD50 per 0.05 ml. Using a syringe and needle, exactly 0.05 ml of the diluted seed virus was inoculated into the allantoic cavity of each egg. Inoculation had to be carried out promptly following preparation of the seed virus, because the virus is rapidly inactivated in the liquid state at room temperature. We found that an experienced two-man team could inject a batch of 250 eggs in a little over one hour after preliminary preparations were made. The inoculating syringe used was a 1 ml tuberculin syringe graduated in hundreds and fitted with a three-way stop-cock and 3/4-inch 24/25-gauge platinum iridium needle. The assembly was connected to a 100 ml syringe containing the diluted seed virus. Here's a picture of it. We were working in a sterility room with positive pressure.

When you opened the door, the air going in went through ducts with ultra-violet lights, which helped to create a sterile atmosphere in the room.

Harden: I also see that you all were wearing sterility garbs like those in operating rooms.

Burruss: Yes, all the work in there was done that way. The eggs you see contained seven-day old embryos. One man took a brush containing tincture of Merthiolate and passed it across the tops of 24 eggs to sterilize them. Then he punched a hole—you see the little punch he is holding—into the egg. A second man picked the eggs up, one at a time, and, keeping them in a straight line, passed them into the needle so that it would inject the 0.05 ml of inoculum into the allantoic cavity. The eggs were placed in another rack where the holes in the eggs were sealed with a mixture of bees-wax and paraffin. The eggs were then returned to the incubator for four more days. In addition, after they finished inoculating the eggs, they made cultures of the seed virus in beef dextrose broth to find if there were any contaminating organisms. The culture had to be negative to proceed to harvesting.

Harden: Let me add one more thing about this picture just for my reference. The syringe is set into a clamp. The needle is horizontal so that the man passed the egg into the needle.

Burruss: Also don't forget there was a three-way stop cock for drawing in more seed virus as it was needed. Here is a better picture showing the stop cock and the size of the needle. We had needle just the right length for inoculating the allantoic cavity. The stop cock, of course, was standard equipment. You bought that from Becton-

Dickinson. They used it for many other things. The needle was put in a flame and sterilized every five or six eggs to avoid possible contamination. Even though we used a disinfectant on the tops of the eggs, when you passed the same needle into egg after egg after egg, there was a risk of contamination. To avoid that, we made the needle red—hot every five or six eggs. Do you have other questions about this part of the process?

Harden: No I think I understand it so far. Let's move on to the next step.

Burruss: At the end of four days, the eggs were taken from the incubator and candled—that is, we put them over a light to see whether the embryo was dead or alive. The dead embryos were discarded, and only the live embryos were taken for harvesting. That was also a two-man operation. Incidentally, here is the list of things we needed for harvesting. It was very important that one technician get all of these things together in advance. Otherwise the embryos might be contaminated as someone went in and out of the sterile room to get the missing items.

Harden: I would like to read this list for inoculating eggs with seed virus into the record: racks to hold the eggs, Bunsen burners, waste pans, wax units, inoculation units, sterile saline solution, 50 ml Erlenmeyer flasks, sponges, gauze sponges, petri dishes, surgical gowns, surgical masks, seed virus eggs, forceps, a mouse-tooth sterile forceps, platinum loops, egg punch, jar of tincture of Merthiolate, the brush, and the syringe with needle.

Burruss: Now for the preparation of the embryo extract, which was, of course, the vaccine. Eggs were harvested about eleven days (255 to 264 hours) following initiation of incubation, provided the culture made when the eggs were inoculated showed no contaminating organism. Eggs to be harvested were removed from incubation and candled, the dead embryos discarded, and those living were taken to the sterility room. If we started out with 250 eggs, we might have ended up with 200 to be harvested. Once the 200 eggs had been brought into the sterility room, one man had the job of cutting the top off the eggs. He used an oxyacetylene torch. The cup holding an egg rotated slowly. It was operated by a motor underneath the bench. As it rotated, the technician held the torch in the right spot, and it cut the top of that egg. That torch was a great invention. Before it was developed, people cut the tops of the eggs off with scissors, and that process would contaminate a great many of them. The torch cut the eggs so much easier and guaranteed that they were sterile. Dr. Hargett sent me a copy of your interview with him. I think he said he suggested this method, and the Rockefeller people in Brazil made some equipment to put it into practice. After the tops were cut off the eggs, they were put in a holding rack. Another technician had a pair of sterile mouse-tooth forceps—a separate pair for each egg. They were placed in stainless steel boxes so they could be removed one at a time. The technician would flip the cap off the top and then carefully take the embryo out and drop it into an aluminum grinding cup. Here is a picture showing how the embryo was placed in the grinding cup. During this entire process, great care had to be given to the following points: only eggs of the specific, single lot destined for this batch of extract were to be

harvested; the egg cap was to be cut at the proper level to facilitate embryo removal; strict asepsis had to be maintained; dead and suspect embryos were to be discarded; and embryos possibly contaminated for any reason had to be discarded. Care was more important than speed. To every three grams of harvested embryo, one gram of sterile distilled water was added immediately after the harvest was complete and prior to homogenization. Extreme care had to be taken to insure that the diluting water was free of contamination. Since the embryos differed in weight, we weighed all embryos together in the grinding cup. We knew what the cup weighed and so could calculate the weight of the embryos. From this figure the technician could add one part water to every three parts embryo by weight. Next the embryos were placed in a grinding cup, a Waring Blender we called it. We put dry ice all around it to keep it cold while we were grinding. Here is a picture of the grinding apparatus. After the embryos were ground, we transferred them from the grinding cup to centrifuge tubes. These were all sterile tubes, and this was a closed system as near as we could get it. You see in this picture how a bell fits over the outside of the centrifuge tube.

Harden: Did you all develop this equipment or were you able to buy it?

Burruss: Some of each. The Rocky Mountain Laboratory had a first class machine shop that made some of the equipment for us. For example, they made these screw-on tops. I can't remember whether they made this whole cup or not. Let's see. It says here we used Waring Blender rotor bases and aluminum cups, Catalog No. 17235, Central Scientific Company, Chicago, with modified tops. We modified the top. The manual notes that care was to be taken that the cups were sterile, gaskets

tight, caps screwed down well, rotating blade units in proper adjustment, rotor bases in good order, and adequate refrigeration with dry ice provided. The homogenization process ran on low speed a few seconds before switching to high speed. The total homogenization period was ten minutes. Refrigeration of homogenization cups had to be carried out to avoid overheating the contents during homogenization with consequent inactivation of virus. This was accomplished by placing a pack of dry ice about the cup, and keeping it intact during the homogenization period. A cotton swab, rubber bands, and clamps was an excellent means to hold the dry ice in position. Things like that we had to invent. Experience soon indicated the quantity of dry ice required. If too much ice was used, the vaccine froze. Care had to be taken to start homogenization at the time the ice pack was applied.

After homogenization, the material had to be clarified. The first step in this process was centrifugation. We transferred the homogenized material to 100 ml heavy Pyrex centrifuge tubes. The tubes were capped with pure gum rubber centrifuge caps. The caps were secured in position with strips of adhesive plaster to prevent them from coming off during centrifugation. Centrifuge tubes were balanced by placing pieces of lead foil in holders when necessary or by use of tubes with water ballast. The homogenized material was centrifuged for 30 minutes at 3,500 rpm in a Size 2 international centrifuge with conical head No. 835. The centrifuge was allowed to stop without braking or with very little braking to avoid shaking up the material. The supernatant fluid, which was the vaccine, was cautiously drawn from the centrifuge tubes into a single Kitasato

flask. Great care had to be taken not to mix the sediment with the supernatant fluid. This picture shows something I invented. It is a long needle to draw off the supernatant fluid. You can also see the chick embryo debris that we didn't want. We drew the supernatant fluid off into a flask using a small amount of negative pressure. The centrifuge tubes had rubber caps. They were replaced by easily removable lids to keep them closed. Once the vaccine had been transferred to the Kitasato flask, we wanted to transfer it from the flask to one-liter storage bottles. Here again we used a rubber tube with a bell over the top of the centrifuge tube to try to keep it sterile. There was a clamp on the tube, so we just ran a given amount.

After this, 2 ml were run into 15 ml Pyrex ampules, which were properly labeled as to lot number and volume. Each ampule's stem was sealed in the oxyacetylene flame. We needed those 2 ampules for potency testing. I might say that doing potency tests in mice was not an exact science. At times, if too few mice were used per dilution of virus being tested, you could get an answer of 100,000 LD50 in one test and 200,000 in another test. But anyway, it all worked. Once we closed those bottles, we didn't open them until we were ready for distribution into ampules and desiccation. But we did need two samples.

Second, three tubes of aerobic culture media and three tubes of anaerobic media were planted with 0.3 ml in each tube. This picture shows us planting the culture media.

Third, all the remaining extract from the flask was run into one or more 1,000 ml Pyrex bottles. That's the big bottle. If the volume of extract exceeded 500 ml, two bottles could be employed. The bottle was closed with a single-hole No. 6 rubber stopper containing 8 ml outside diameter Pyrex glass tube called a C unit. It was positioned at an angle of 110° and contained a cotton air filter. After this stopper was well in position—we employed a special clamp—the medicine stopper and glass stem was painted with paint to prevent any air from getting in. Then the stopper was anchored securely in place with adhesive plaster strips. The mouth of the C unit was covered with short 3-inch section of 1/4 inch by 1/16 inch rubber tubing closed near the terminal end with the screwed clamp. Now here is the special clamp we used. We told the engineer of the machine shop to make us something that would push the stopper in tight, tighter than we could push it in. As I said, they could make anything like that within reason that we wanted and much more complicated than that.

The next step was to freeze the vaccine, or embryo extract as we called it, until we were ready to distribute it into ampules. In this picture you can see the special pan we made to rotate the bottles in while we froze the vaccine. The dry ice and alcohol would overflow, and we caught it in this pan. This is the main container to hold the dry ice and alcohol. That's a very cold mixture, dry ice and alcohol. The bottle had to be rotated slowly. There's the crank. The technician turned it slowly to freeze the vaccine on the sides of the bottle rather than in one big cake at the bottom. The purpose of this procedure was to facilitate thawing when we wanted to use it. If it was spread all over the bottle it would thaw in

much less time than if it was in a big cake. This picture shows our dry ice storage refrigeration cabinet. This was something that Dr. Hargett and I thought of and got the machine shop people to make for us. We wanted a ready supply of alcohol at dry ice temperature. We had a little tank in this dry ice refrigerator that kept a certain amount of alcohol cold all the time. Any time we needed it quickly, we just opened some of these valves, and it came out.

When the bottles were frozen, the glass tube we called the C unit was open so that air could enter through a sterile, cotton filter to equalize the pressure. The glass tube of the C unit had previously been drawn out with a torch so it would be easy to cut when we wanted to use it. It's small at one end, so it was easy to slip a rubber tube over it.

Here is a picture of where we kept our storage bottles of vaccine. They were kept at dry ice temperature. Desiccated vaccine was kept in a refrigerator at minus 25° C. The space under dry ice storage was limited, however. We had enough for bulk frozen vaccine only. We did not have to keep desiccated vaccine there. When we wanted to make a lot of vaccine, we were ready to take several of these storage bottles and join them together for a lot of desiccated, finished vaccine.

These bottles in the storage cabinet, of course, had to pass the sterility test and a satisfactory mouse potency test. If they did that, we were willing to use them to make a lot of finished vaccine. We would take from one to five bottles of our acceptable, frozen vaccine from the dry ice storage cabinet and thaw them. It

took about a half hour in 37°C water to thaw the frozen vaccine so that we could use it.

The liquid extract was then transferred into the dispensing flask—a 1-liter Kitasato flask, or a 2-liter flask with two or three inlets in the stopper, or a 4-liter flask with six inlets. In making these connections, the end of the glass tube in the extract bottle was broken off at the constriction and the inlet tube of a dispensing flask connected. Once the vaccine was in the flask, we were ready to dispense it into ampules. We made four different sized ampules of vaccine: 5-dose, 20-dose 40-dose, and 100-dose. Most of our demand was for the 5-dose and 20-dose sizes. Once you opened an ampule and rehydrated it, it had to be used immediately. That is, if you had only one person to vaccinate, you would have to use a 5-dose ampule to inject the person and throw the rest away.

Harden: Just how long would it last? A few hours?

Burruss: Oh, it was good for an hour or so. As long as it would take to inject the people. Our instructions said to use it immediately. I forget exactly what limit we placed on it, but it was good for only about one hour.

In making a lot of finished vaccine, we would dispense 0.5 ml for 5 doses, 1 ml for 20 doses or 40 doses depending on the titre. And 5 ml for a 100-dose ampule. Using the burette in this picture, we could easily measure 0.5 ml. Of course, you couldn't touch the mouth of the ampule. So we used ampules plugged with cotton wrapped around a wooden applicator for easy handling. This arrangement was also our invention. And when I say our, I can't remember who

thought of it, Dr. Hargett or I. It was a very simple invention. A lot of those things were simple, but they sure worked well. It was much better than handling the ampules with rubber gloves. Even if you used sterile rubber gloves they wouldn't be sterile very long.

Before we used these ampules for our vaccine, however, we tested each one empty, as it came from the factory. A small percentage of them had pinpoint holes and were unsatisfactory. We had to put every ampule on a special unit and test them with a high-frequency coil. Any ampule having a hole in it showed up as a different color. If it was tight, it gave a bluish color, but if it had a hole in it, you could see a yellow line going through it. Once we had discarded any faulty ampules, we were ready to fill the rest, after they had been washed and sterilized. Here's a little table we used for dispensing vaccine extract into each ampule: 0.5 ml with 50,000 minimum LD50 per 0.03 ml for a 5-dose ampule; 0.5 ml with LD50 of 200,000 or 1 ml with a minimum LD50 titre of 100,000 for a 20-dose ampule; 1 ml with a minimum LD50 titre of 200,000 for a 40-dose ampule; and 5 ml with a minimum LD50 titre of 100,000 for 100-dose ampule.

In this picture you see a man running the required amount of liquid vaccine into the ampule. And I might say there was a trick to this. When you ran this down, a drop tended to collect on the end of the needle, but if you gave this pinch valve just a little flick, it would blow that drop off. The process required care because we didn't want the vaccine to remain on the stem of the ampule if we could help it, since that made it harder to seal.

This vaccine was freeze-dried: you froze it first, and then you dried it from the frozen state. For the freezing we had more equipment. The first piece of equipment needed was what we called the surface tension breaker. We wanted to distribute the vaccine thinly around the sides of the ampule. Thus we put them on this surface tension breaker which rotated the ampules slowly until a thin film of vaccine was evenly distributed along the sides of the ampule. This was another machine Dr. Hargett and I invented and asked the machine shop at the Rocky Mountain Laboratory to make.

Next we had a shell freezing machine which used a tank of alcohol and dry ice. The ampules were rotated with rotors to freeze the vaccine thinly on the sides of the ampule. And as soon as they were frozen, they were placed in a test tube rack and immersed in dry ice and alcohol to keep them frozen.

Periodically these ampules were transferred to a refrigerator where loading on a manifold took place. That refrigerator was kept between -20 and -25°C. Once the 600 ampules were shell frozen and placed in the refrigerator, they had to be connected to manifolds for the drying desiccators. They were inserted into rubber tubes on this manifold, which had approximately 96 outlets or inlets. In connecting the ampules to the rubber sleeves of the manifold, we greased each rubber sleeve with castor oil. The excess oil was blown out with a pressure gun. The castor oil had two purposes. It made it easier to insert the glass ampules into the rubber sleeves, and it also tended to prevent leaks. We had a horizontal and a vertical manifold. When two of these manifolds were loaded in the refrigerator at —25°C, we placed wooden covers around them, which I designed and had the

engineering shop construct. These covers were important because we had to move fast in transferring the manifolds containing the frozen ampules to the high-vacuum pumps for drying. If the frozen vaccine melted before it dried, a poor looking, unsatisfactory product resulted. But if it stayed frozen until it got under the high vacuum and started to dry, the desired product was produced.

When the manifolds containing the ampules of vaccine were connected to cold traps immersed in alcohol and dry ice and put under high vacuum, the liquid from the vaccine condensed inside the cold traps and later on collected on the bottom. Both the vertical and the horizontal manifolds were desiccated at the same time. While that was being done, two more were being loaded in the cold room. Eventually all six were loaded. For quite a few years we did not have those wooden covers to trap the cold air from the refrigerator when we bought the vaccine out to connect it to the vacuum pumps. Occasionally, the frozen vaccine would melt before it started to dry and, of course, that would spoil it. Eventually we thought about having these covers made to trap that cold air to keep it frozen during those critical first 5 or 10 minutes. For the 5-dose and 20-dose ampules, 2 to 2 hours were required to complete the desiccation of the vaccine. For the 100-dose ampules, 4 to 5 hours were required. The reading of the vacuum CENCO McLEOD gauge during the last hour had to be 1.5 microns of mercury or better and a final reading of 1 micron or better needed to be obtained.

After the vaccine was dry, the next step was sealing. Nitrogen was introduced into the vacuum system prior to sealing through an inlet provided for this purpose. We had some tubes and inlets so that we could take dry nitrogen and

introduce it into the entire system. The ampules were then removed from manifolds without the use of clamps. They were rapidly inspected to see that the contents were in acceptable condition, that no castor oil remained on the lower part of the stems, and that the containers were in a good state. Then the stems were sealed about 3/4 inch from the tips in the oxyacetylene flame. Care was taken to secure a tight seal. Spirals and bubbles in the seal were avoided insofar as possible. To accomplish this task, we all virtually had to become glass blowers.

Sealed ampules were promptly and carefully inspected with particular attention to any defective containers, defective seals, extraneous materials—particularly castor oil within an ampule—and verification that the contents had been properly desiccated. At this point, labels were applied to each ampule. Great care had to be taken that the labels were correctly and completely filled in except for the expiration date, which was filled in at the time of shipment. Here is a sample label for the vaccine. It says that the content is living dried culture 17D vaccine prepared from infected chick embryo and gives the lot number. It also gives directions for preparing the vaccine to be administered: “Dissolve contents in 20 cc of saline.” That would be a 40-dose ampule. The expiration date is also shown. The dose is 0.5 cc. “Refer to printed instructions.” The inspected and labeled ampules were put into numbered storage boxes and placed in numerical order in a special -25°C or colder storage refrigerator. We prepared a card index to show which lot was in which box. Here is a picture of a vaccine storage box we designed. It didn’t take much effort to design that. It was made in the carpenter’s shop at the Rocky Mountain Laboratory. If we wanted a particular lot of vaccine,

it was very easy using the index card file to go to the refrigerator and bring out the boxes that were already numbered.

Harden: How many ampules would this box hold?

Burruss: Each box would hold 120 ampules of 5-dose, 20-dose, or 40-dose vaccine because the ampule size was the same for all of those. For the 100-dose size we had a bigger ampule, so the box would hold fewer. Before the vaccine could be shipped, there were final sterility, safety, and potency tests from ampules in each lot of vaccine. We had different forms for these. YFU-8 was used for recording results of control *tests* on finished vaccine. A copy of “Yellow Fever Vaccine Directions for Dilution and Administration” was included with the vaccine we shipped. The sterility testing department of the Rocky Mountain Laboratory conducted the final sterility tests. They planted 3 ml of vaccine from each of 13 ampules selected at random into each of four tubes of media. Half of these tubes were incubated at 37C and the remaining half were left at room temperature. After an incubation period of seven days, the results were entered on a form. The vaccine was considered free of bacterial contamination provided all culture tubes showed no growth. Vaccine was regarded as contaminated and was discarded if two or more tubes showed growth. If only one of the tubes showed growth, the test was repeated, using the same number of ampules originally submitted. If the same type of organism appeared in any of the second set of tubes planted, the lot of vaccine was discarded. On the other hand, if no growth appeared in any of the second set planted, the lot of vaccine was considered sterile. We also had a guinea pig safety test in order to detect possible contaminants that would not grow in the

culture media. Every lot of yellow fever vaccine was tested by inoculation into guinea pigs. Two normal male guinea pigs in good health, weighing 400-to-500 grams, were each injected intraperitoneally with 4 to 5 ml of vaccine. The guinea pigs were observed for a period of fourteen days after inoculation, and their temperatures were taken once daily. A temperature of 39.8°C was regarded as fever and an indication of illness. All animals were autopsied at the end of post-inoculation and observation period. Findings were recorded on form YFU-36. The test result was regarded as satisfactory provided both animals showed no significant clinical manifestation during the fourteen-day observation period. If both pigs showed significant reactions, the vaccine was discarded. If only one animal showed a significant clinical reaction, the test was repeated using three test animals. In the repeated test, if more than one of the three guinea pigs showed a significant reaction, the vaccine was discarded. In addition to the sterility and safety tests, we conducted potency tests. Potency of the finished vaccine was determined by the intracerebral inoculation of serial 10-fold dilutions into suitable numbers of acceptable white Swiss white mice 35-to-42 days of age, weighing 16-to-20 grams each. An ampule of desiccated vaccine was rehydrated in normal physiological saline solution to 1/10 of its labeled volume in order to obtain undiluted vaccine. Eighteen to twenty-four mice per dilution were inoculated, depending on the quantity mice available. Tuberculin syringes 1/4 cc capacity were used. These were fitted with 27-gauge 1/4-inch needles with guards which allow only 3-to-3 1/2 mm of the point to penetrate the mouse's brain. That was my invention. I wrapped a thin copper wire around the needle, leaving only 3-to-3

1/2 mm exposed. 0.03 ml was injected into each mouse while under light ether anesthesia. The needle was inserted at a point midway between the eye and the ear and a little to one side of the mid-line. Here is a photograph of this process. No antiseptic was used at the site of inoculation nor was the hair removed. The mice were placed in numbered jars in groups of six. For each jar a record card was made out. The information recorded daily on the record card throughout the 21-day observation period referred to the group as a whole and not to individual mice. The key to the symbols used was printed on the reverse side of the card. At the end of the 21-day observation period, the mice record cards were reviewed, and from the number of deaths and survivals for each dilution, we could determine the LD50 titer per 0.03 ml.

Harden: Could you tell me what happened to the yellow fever unit after the war ended?

Burruss: In 1952 Dr. Victor Haas, who was the Director of the National Microbiological Institute of which the Rocky Mountain Laboratory was a Division, decided that the yellow fever vaccine production should be transferred to the private sector. The main reasons were that funds expended and the laboratory space released could better be used for other work. Arrangements were made with the National Drug Company's Biological Division in Swiftwater, Pennsylvania, to take over the yellow fever vaccine production. All yellow fever vaccine equipment at the Rocky Mountain Laboratory that could be moved was sold to National Drug. I worked for them one year setting up the yellow fever production laboratory and training the necessary personnel while producing enough vaccine to fill a U.S. military contract. In the fall of 1953 I returned to the National Microbiological

Institute and worked in the Pyrogen Section of the Laboratory of Biologics Control until my retirement in December 1970. Later on the above names were changed to Laboratory of Control Activities in the Division of Biologics Standards.

Harden: Thank you, Mr. Burruss, for talking with me.