Of Computers, Chemostats, Coulter Counters and Centrifuges

The beginning of my career as a laboratory scientist can be traced back, most directly, to a sophomore course in American Literature at Iowa State College in 1956. That, in itself, is most astonishing because, when I entered college six years after graduating from high school I still harbored a vivid memory of how much I hated American Literature. So, this course must have been the best available choice among some less than exciting core electives. To my surprise, however, the class was a delight and the professor was inspiring. He had done his doctoral research on Nobel laureate Sinclair Lewis and he included Lewis' novel, *Arrowsmith*, as one of the readings in the course. That novel set me on a path that ultimately took me to a laboratory in Gainesville, Florida, which will be the primary setting of the following narrative.

Arrowsmith follows the life and career of a physician in the early 20th century. He became a research microbiologist and achieved some fame as a result of his work. When I read the book, I was an undergraduate and contemplating a career in medicine. At that time, I was in need of a part time job—and some practical experience—so I checked to see if any professor in the Department of Bacteriology might be looking for a part time laboratory assistant. My timing was perfect. Prof. William Lockhart had just received a grant with money budgeted for a technician. I interviewed with Dr. Lockhart and got the job—mostly, I expect, because I was older than most undergraduates and was an army veteran—and even though I had never yet taken a course in bacteriology.

I soon learned that technicians in bacteriology laboratories do not do very exciting work. All dishes had to be hand washed—and there were always a lot of them. Because cultures of bacteria grow on special media, the media had to be prepared, sterilized and maintained in sterile conditions. So, my initiation into the world of bacteriological research introduced me to Thomas Edison's famous statement that invention is 99% perspiration and 1% inspiration. My job as a technician was to provide most of the perspiration.

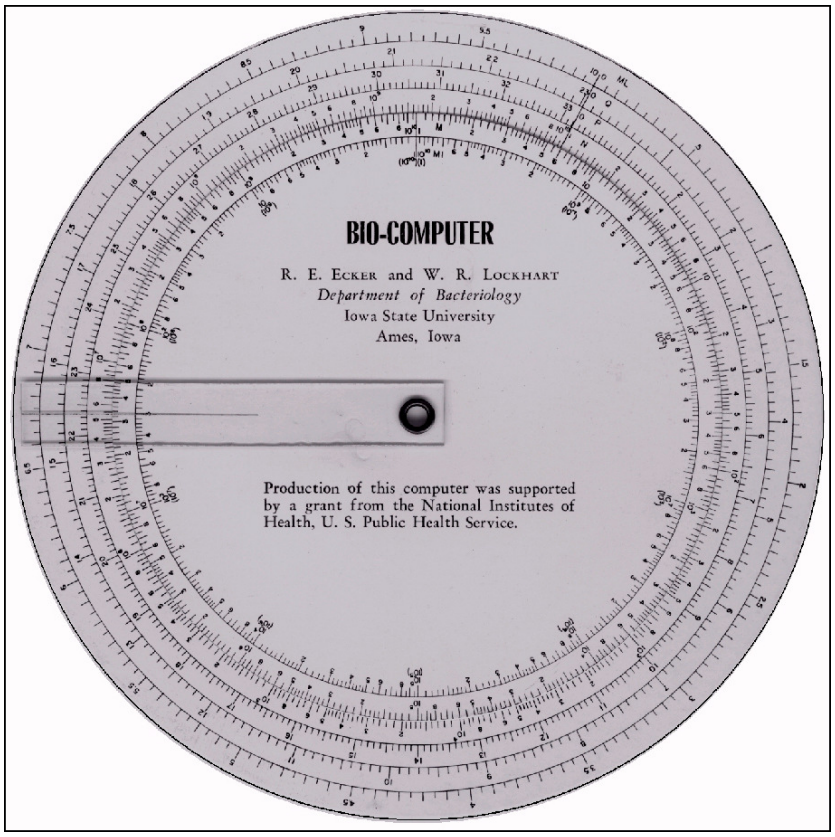
However, Dr. Lockhart was a patient mentor and an exceptional educator. He must have seen in me the potential for something more than washing dishes, for he began to train me in the skills needed to manipulate cultures of bacteria in the laboratory. I learned fast and soon began to assume responsibility for much of the day-to-day operations in the professor's research program. And, with that responsibility, I also began to do some experimenting on my own, particularly in regard to some of the equipment and methods we were using to carry on the research. While this was going on, Dr. Lockhart began encouraging me to continue as a graduate student in his laboratory rather than going on to medical school. I ultimately agreed and, by the time I finished my undergraduate work (with a degree in bacteriology), I had already co-authored three methods papers in the scientific literature—two of them as senior author. Clearly, I had found a home in the laboratory.

As a graduate student, my thesis research involved growing bacteria under different culture conditions, analyzing the results and drawing conclusions about how those conditions influenced the parameters of growth. One of the key elements of those analyses was counting the size of bacterial populations, which can become very large. Typically, my cultures would contain up to more than a billion bacteria per milliliter. Because of this, I had to undertake the regular calculation and communication of large numbers. However, at the time, there were no instruments available to do such calculations…so I invented one.

In high school during the 1940s, I was introduced to a now-obsolete instrument called the slide rule. I learned how to manipulate it and made regular use of mine right up to the time I was

in graduate school. The problem with the slide rule was that it only gave you the root value of a number. It could not tell you where to put the decimal point. Here is a personal story to illustrate that problem. Before I started to college, I worked in the family business, a greenhouse. That summer, we built several new houses and we needed to fill the growing benches with dirt. But how much dirt? I measured the dimensions of each bench and used my slide rule to calculate the volume. It gave me the root number 652—but did that mean 6.5, 65 of 652 yards. It seemed to me that 6.5 yards (about a truckload) was too little, so I anticipated having to order ten truckloads to fill each bench. However, to my surprise, the first truckload completely filled one bench. My decimal point was in the wrong place.

To eliminate that kind of problem, I needed an instrument that was basically ten slide rules long, which was possible if you connected the scales in a circle and diminished their length. The diminished length was not a problem because I usually needed no more than two significant figures in my calculations. Also, I wanted it to be able to inter-convert base 10, base 2 and base e logarithms. The final version of this computer is shown at the right. Basically, it consisted of two printed paper discs attached at their centers to a plastic indicator arm.



Although I produced my initial, rough draft, copy of the computer just for my own use in the laboratory, the idea began to grow legs and Dr. Lockhart, now my thesis advisor, stirred up some grant money to have it produced in quantity for distribution to interested colleagues in the field. We placed an article in a trade journal, describing the device and announcing that it could be obtained by sending us a postcard…in the same way that interested readers would request reprints of articles from scientific journals. The response was huge. We received hundreds of requests from all over the world.

Meanwhile, I continued my thesis research and began to consider what I wanted to do after I received my doctorate. Professor Lockhart recognized that the classical education in bacteriology I had received at Iowa State had done little to prepare me for the really cutting edge of work in the field—namely, in molecular biology—and that I had not, and could not have, become a really good experimentalist in his laboratory. His recommendation was that I seek a postdoctoral fellowship in the laboratory of an established molecular biologist. He further suggested that I consider the laboratory of Dr. Moselio Schaechter at the University of Florida College of Medicine in Gainesville.

At about that time, with perhaps six months remaining before graduation, I attended a meeting of the American Society of Bacteriologists in Philadelphia. One day while I was there, I entered the elevator at the headquarters hotel and, eyeing the nametags of my companions in the car, I noticed on one of them the name "M. Schaechter, University of Florida." I introduced myself to him and asked if he had some time when we could talk. He paused for a moment, looked at my nametag and then said, "Oh. You're the guy that invented that Bio-Computer." I acknowledged that I was and, when we got off the elevator, we arranged a time and place for an interview. A couple of months later, I received the offer of a postdoctoral fellowship in the Department of Microbiology at the University of Florida College of Medicine. Whether the Bio-Computer was instrumental in my qualifying for that fellowship I do not know, but I'm sure it helped grease the skids.

By the time I finished graduate school, we had three kids—all of them born in Ames—so our move to Florida was a major family affair. We left for Florida in early March 1961, with all of our worldly possessions in, on top of or behind (in a trailer) our new 1960 Ford Falcon station wagon. It was a perfect time of the year to experience the contrasts between Midwest weather and weather in the Sunshine State. A few days earlier, we had made a visit to Myrna's parents in southern Minnesota. When we left there, we had to dig our snowbound car out of their driveway. The photo on the right shows us enjoying the contrast at the Florida welcome center as we entered the state a few days later.

When we arrived in Gainesville, we discovered that "March Madness" had got there before us. The state high school basketball championships were underway at the university and there wasn't a motel or hotel room available in the community—except at the Hotel Thomas, a beautiful old antebellum edifice right in the middle of town, surrounded by at least an acre of spectacular gardens. On the left is a photo looking up a garden path toward the hotel. Clearly, we were "not in Kansas any more."

We found accommodations at the hotel and settled in. As I recall, the price wasn't that unreasonable. I expect that vacancies at the Hotel Thomas during "March Madness" had less to do with the cost than with the fact that it was not the sort of place where exuberant basketball fans could expect to let off steam without invoking disapproving stares from the mostly-aging inhabitants. In fact, that aspect of the place came into play in a big way when we went to the dining room for dinner that evening.

The dining room was large, ornate and filled with people who could have been our grandparents. After we were seated, I took one look at the menu and knew we had trouble. We were running short on cash because I had lost our travelers checks at one of our stops on the way down and were still waiting for a refund. Nothing on the menu came within the limits of our

limited resources. We simply couldn't afford to eat there. But there we were. It was a most troubling moment—until Eric came to the rescue. Eric, our youngest, was just a year old and, as if on cue, he started crying, attracting attention from the diners around us. That was all I needed to pack up the crew and head for the exit, explaining to the hostess that we didn't want to disturb the other patrons. From there, we took a walk on a balmy evening to the business district a few blocks away, where we found a place for the whole family to eat for less than ten bucks.

I don't think we stayed in that hotel more than one or two nights. Dr. Schaechter had scoped out some possible houses to rent and we moved into one almost immediately. In most respects, it was a pretty typical Florida home—concrete block construction on a slab, with jalousie windows and space heater. In other respects, however, it was anything but typical. It had once had a carport with an entrance off the kitchen. Now, the kitchen had been expanded to include the area of the carport. Another rear exit now opened to an enclosed breezeway that led to a mother-in-law apartment of the same construction as the rest of the house. We got a lot of house for our rent, which was $125.00 per month. For us, after living for the last six and a half years in 500 sq. ft., it was palatial—and the yard seemed almost boundless.

From this point on, this will become a two-track narrative. One track will cover my life at the medical school; the other—mainly photographs—will cover the life of our growing family during the years we lived in Gainesville. My earlier narratives were mostly just about family life. However, as I considered writing about our Florida experience, it seemed to me that the family members in those days saw me disappear regularly—sometimes at odd hours—without ever knowing what was really happening when I announced "I'm going to the lab." So, I will try to describe some of the details of my days at the lab, hopefully without becoming disgustingly technical. The narrative will alternate among several settings and experiences.

In my graduate work, I had grown bacteria and observed their growth patterns under different conditions. Now, I was about to embark on a project asking questions about what was going on inside those cells—at the molecular level—as they were growing. In particular, I was going to look at an intracellular component called a ribosome, which was considered to be the site of protein synthesis in the cell. Dr. Schaechter had done work earlier suggesting that ribosomes seemed to function at constant efficiency; that is, the rate of protein synthesis for each individual ribosome appeared to be constant. However, there was no direct evidence to establish the validity of that hypothesis. My project was to find the direct evidence required to substantiate it.

What we were saying was, if you need a higher rate of protein synthesis, then you will need more ribosomes—and if ribosomes function at constant efficiency, then there should be a direct relationship between the rate of protein synthesis in a cell and its content of ribosomes. So, I needed to design experiments that would tell me if the rate of growth of bacterial cells (for the cells are made up primarily of protein) is proportional to their ribosome content —and that's what I set out to do. And if you are inclined to ask why we care what's happening inside a bacteria cell, you need to be aware that the basic molecular mechanisms in all cells are essentially the same. What you learn about the molecular biology of bacterial cells will be pretty much applicable to all cell systems. And bacterial systems are a lot easier to work with, particularly in regard to regulating their rates of growth.

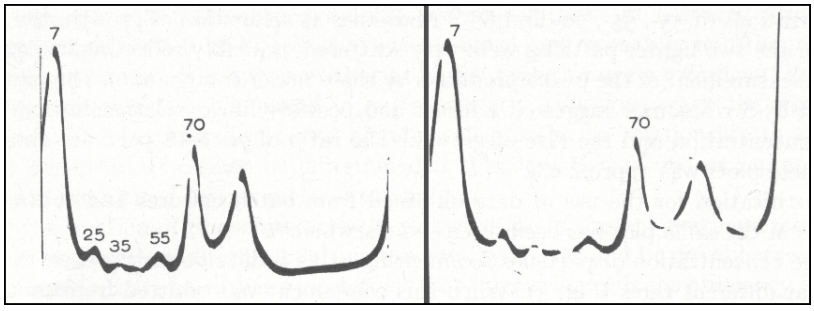
Bacteria—at least the kind we were growing—are extremely adaptable creatures. They will grow in a large variety of different media. In each of those media they will grow at a slightly different rate, depending upon the availability of essential nutrients. At the least, they will grow in a chemically defined medium containing no more than an energy source—typically glucose—

some inorganic sources of nitrogen, phosphorous, sulfur and several essential minerals. In this medium, the maximum sustained rate of growth was about one generation per hour. That is, on the average, it took each cell an hour to double in size and divide into two new cells. You could increase that rate of growth by adding nutrients (vitamins, amino acids) to that basic medium, with the rate increasing as you increased the variety of nutrients added. The maximum rate we could obtain in this way was about three generations per hour (one cell division every twenty minutes).

So, we could manipulate the growth rate of our cultures over a three-fold range by simply growing them in different media. However, to give the experiment the validity we required, we had to be able to grow cultures at even slower rates, preferably as near zero as possible. That we could do, in fact, with a little help from a apparatus called a chemostat, a continuous culture device that regulated the growth rate by changing the flow of medium through the growth chamber. However, before I explain a bit about how chemostats work, I need to fast forward for a moment to the process of analysis, by which I was able to determine the cell's content of ribosomes at any particular growth rate.

Ribosomes are large, complex molecules. In this case, however, "large" is a relative term, because all molecules are small in relation to the size of the cell, which itself is very small—perhaps about one micrometer (or 1/1000 of a millimeter) in length. So, if I wanted to know how many ribosomes were in a cell, I was not going to be able to count them under a microscope. I needed to separate the ribosomes from the rest of the constituents in the cell and then measure their relative prevalence. That was possible to do—but not simple.

First, I had to concentrate the cells using a laboratory centrifuge and then break them open to get the ribosomes out where we could work with them. I won't go into that process, but it was an established method and it worked well. After this procedure, I had a small volume of highly concentrated ruptured cells. The method I used to analyze that soup for its concentration of ribosomes involved another centrifuge—a very specialized one, called an analytical ultracentrifuge. It was capable of spinning samples in a rotor that could be spun at over fifty thousand revolutions per minute. In addition, it was able to take pictures of the sample as this intense centrifugal force drove the various constituents at different rates toward the bottom of the sample chamber. The adjoining photograph shows the patterns produced by this camera at two different times after the centrifuge reached its running speed—with the centrifugal force directed from left to right.



It was already known that the four peaks to the right of the one labeled "25" were ribosomes—and it had been shown that the area under each peak in these patterns represented the concentration of the cellular component represented by that peak. The peak labeled "7" constituted the non-ribosomal cytoplasm of the cell—primarily protein. So, the area under this peak represented the amount of protein being synthesized by these ribosomes. We will see a bit later how these relationships can be used to test the validity of the theory being investigated. Meanwhile we will move down the hall from my research laboratory to the departmental

conference room and to the classroom and teaching laboratory where second-year medical students were working toward credit in the school's course in Medical Microbiology.

Although I was appointed to a postdoctoral research position in the department, that title did not immunize me from responsibility for teaching in the department's class for medical students. As far as the departmental chairman was concerned, I was a member of the faculty and the faculty taught the course as a team. It was in the conference room that we met together as a team to discuss teaching assignments and to formulate examinations. We were not a large faculty—perhaps ten at most, even with postdoctorals and people with joint appointments—so the faculty culture in our department was a rather intimate one. That intimacy was facilitated by the common practice of having a brown-bag lunch together in the conference room. Those lunches were great opportunities for getting better acquainted with our colleagues and for withdrawing a bit from the more serious business of research and teaching.

One of those colleagues was a tall medical resident named Dan Tucker. He was in the Department of Medicine, but was doing research in our department. Two stories involving Dan Tucker are worth relating. The first happened at lunchtime. I could write a whole book on the interesting contents of the brown bags that appeared on our conference table every noon, which often had fascinating stories of their own. On the particular day in question, Dan was sitting next to me at the table, eating some sesame-seed crackers. Suddenly he announced to the assemblage, "Hey! One of my sesame seeds is moving." I looked over and, sure enough, one of the dots on the top of his cracker was walking off onto the table." Insects in Florida were a way of life. This one had chosen a fascinating time and place to reveal itself.

My second memorable experience with Dan was more dramatic. One morning I answered the phone in my office and heard a frantic Myrna on the line telling me that Eric (age 1-1/2) had just gone into the storeroom and drunk some turpentine that had been used to clean paint brushes. The victim and the storeroom door are shown at the right. Typically, that door was secured, but with two older siblings, that obviously couldn't always be guaranteed.

I told her to hold on and stay calm till I could get some advice. I put down the phone and rushed into the hallway to find a physician among my colleagues. In the hall, I encountered Dan Tucker, MD. After hearing what had happened, Dan advised me to have Myrna avoid making him vomit and that we would be right there. Then he said, "Let's go!" We rushed down three flights of stairs, found my car in the parking lot and took off like scared rabbit. I lived several miles from the medical school and I'm sure I broke a lot of traffic laws getting there. When we arrived, Eric was lying on the sofa looking drowsy. Dan said that was to be expected because turpentine can be anesthetic. He checked the child's vital signs, declared him to be out of immediate danger, picked him up and we headed back to the car. Myrna had to stay with the other kids. I promised to keep her advised.

The trip back to the university was considerably less hectic and by the time we arrived at the emergency room of our teaching hospital Eric was sleeping. The emergency room staff took the boy into an examination room and set about pumping his stomach. Dan returned to his lab upstairs with my heartfelt thanks. I can't think of a more traumatic way to introduce a kid to the

emergency room than to have a bunch of people he doesn't know whisk him off to a little room, shove a tube down his throat and flush out his insides—repeatedly, until they don't smell any more turpentine. He may have been only a year and a half old but he never forgot that experience. Whenever he had to be taken back to the emergency room—which his adventuresome spirit made rather frequent—he would start hollering when we drove into the parking lot.

While we are on the subject of kids and the teaching hospital, I should point out that the families of the medical school faculty were all patients of the medical school staff. Having a new teaching hospital in a relatively small town was a significant concern to the local medical society. So the school had had to agree not to take any patients but referrals—individuals that physicians in the area felt had sufficiently severe conditions that they required treatment by the cadre of specialists at the medical school. This meant that the house staff of the school (particularly residents and interns) would have no opportunity to see patients presenting with routine kinds of conditions—except for the families of the medical school faculty. So our children became treasured patients for the residents in the Pediatrics Department, who had little other opportunity to see kids on a routine basis.

Our medical school was a very new one. The first class, I believe, had graduated just the year before I got there. And the classes were still quite small—sixty-four as I recall. So, both the lecture hall and the teaching lab could accommodate a class of that size and we didn't have to divide up the class for lectures or lab sessions. Medical Microbiology was taught to second-year students in the fall term. The course involved a fairly intensive schedule of lectures and laboratory work. All faculty members attended all lectures and participated in supervising all of the laboratory exercises in the course.

I did not like lecturing to medical students. The labs were somewhat more tolerable. The reason for my distaste for the lectures was that I discovered early on that these students approach medical education with little curiosity. As they began their second year, they were only interested in the "what" and not the "why." They wanted facts—and nothing but the facts. To facilitate that quest, they divided themselves up into groups called note takers. Each week, one of the groups would occupy the front row of the lecture hall and busily record on paper the material being presented in the class. Later they would get together, compare notes and publish an official version that would be distributed to the whole class. As I saw it, it was a perfect way to guarantee uniform mediocrity.

The teaching labs were more fun. However, there was seldom enough class time for the students to become proficient in the various procedures and methods they had to learn. So, the lab was open every evening during the week—and each faculty member was assigned one evening to be present there to answer questions and assess progress among the students. For me, those evenings were the most rewarding teaching experience in the Medical Microbiology course. It was where you really got to know the students and had a real opportunity to help them find their way through the subject matter.

We moved to Gainesville in March. For the family—particularly the kids—it was an adventurous new environment. The house we moved into had large azalea bushes in the yard that were in full bloom when we arrived. The kids were reveling in the overnight transition from winter in Iowa to the semi-tropical spring of northern Florida. And they couldn't get enough of the spacious expanse of grass that was now theirs for whatever activities they might dream of undertaking. It was an unbelievable change from the postage-stamp yard in Ames that had been

their playground ever since they were born. In addition, we were living was on a dead-end street, so car traffic past the house was minimal.

The group of photos on the right were taken while we lived in that house. In the first picture can be seen the unique fence that enclosed the back yard of the house. The fence was made of interlaced red brick, and it circled all the way from the back of the mother-in-law apartment to a gate next to the side of the house. Sue was in kindergarden when we moved to Florida. Her school was near-by and the church we first attended met in its multipurpose room.

Jacksonville was only perhaps 70 miles from our home, so the zoo there became a popular place to visit. The kids were enthralled with the crowned bird and, for some reason, gave it the name "Uncle Charlie" which has stuck to this day. The monkey island was a favorite spot and we always came equipped with some bananas to draw their attention.

The trip to Crescent Beach on the Atlantic coast was slightly shorter and it was a favorite venture on Sunday afternoons. We will always remember the wonder we felt on our first view of the ocean. Driving over a slight rise after crossing highway A1A, suddenly there it was—a wide beach and endless water. You could drive for miles on the beach and stake out your own territory, even on holidays.

Back in my laboratory, I was faced with the need to cultivate bacterial cultures at as many different rates as practicable. As I indicated earlier, I could obtain a number of faster rates in batch culture by varying the medium used for cultivation. However, for slower rates I needed a different technique—and I had to have larger volumes. This technique was continuous cultivation using a chemostat. In a chemostat, bacteria are cultivated by continuously flowing culture medium through the growth vessel while maintaining a constant volume. Different rates of growth were achieved by varying the flow of fresh medium into the culture vessel. The medium had a limiting concentration of one of the nutrients (in this case, glucose), so the bacteria could only grow as fast as this limiting supply of nutrient could support. The slower the flow of the medium through the vessel, the more limited the availability of the nutrient—and the slower the growth of the bacteria. Using this apparatus, we were able to achieve growth rates as low as 0.06 generations per hour, or one cell division every fifteen hours.

The reason I needed larger volumes using the chemostat was because, at slower growth rates, the bacterial cells had fewer ribosomes (or so we postulated) and we needed higher concentrations of cellular material to be able to find the ribosomes using our analytical process. In batch cultures, I typically grew the bacteria in one-liter bottles containing half a liter (about a pint) of culture medium. This volume provided ample amounts of material at any of the achievable growth rates. My chemostat was a five-liter glass cylinder with the culture volume maintained at anywhere from 1.5 to 4 liters, depending on the growth rate of the bacteria. However, these larger volumes introduced another technical problem in the process, and it required the use of another unique kind of centrifuge.

I indicated earlier that I harvested the cells from my batch cultures using a laboratory centrifuge, in which containers with small to medium volumes of material could be rotated at a few thousand revolutions per minute, causing the bacteria to sediment in a reasonably short time. However, larger volumes required the use of a continuous-flow centrifuge. This device was essentially a brass cylinder about three inches in diameter and rotating on tubes at the top and bottom through which the culture medium could be fed—from the bottom. When the rotating cylinder reach sufficient speed—somewhat greater than that of the laboratory centrifuge—the culture media were piped through it at a sufficiently slow rate that the bacteria would be sedimented by the time each initial volume had reached the top of the cylinder. At the end of the run, the bacteria could be washed off the inside of the cylinder and prepared for analysis in the same way as the sediments from the batch cultures.

Although I have now described all of the basic techniques—and most of the equipment I used in pursuing answers to the questions that defined my research project—I need to emphasize that the conduct of the work was a lot more time-consuming than my descriptions may have suggested. A lot of preliminary work needed to be done to get to the point of actually undertaking an experiment. Equipment had to be designed. Parts had to be ordered. Custom designs had to be fabricated in the medical school machine shop. And, sometimes, my "best laid plans" simply went astray—not because they weren't good plans, but because of circumstances over which I had no control. The most frustrating of those circumstances came from the laboratory of the virologist next door.

Like people, bacteria can be infected by viruses. When those viruses infect bacteria, they are called phages. The guy next door was not a particularly good scientist, so he tended to play around with a lot of different kinds of viruses, apparently hoping to find something worth pursuing. Perhaps it was inevitable that he would venture into bacterial virology, but why he chose a phage that would infect my particular strain of bacteria, I do not know—nor to I have

any idea why he wouldn't have reasoned that saturating his neighborhood with an atmosphere filled with the little buggers might have some negative effects on my cultures. All I know is that, one day I looked at my chemostat and it suddenly cleared up and was foaming all over the place. It was a baffling mystery until I discovered what was going on next door. It took some weeks for me to rid my lab of the plague and insulate it from the vagaries of my viral colleague.

Returning for a moment to the departmental conference room, where the faculty assembled each day at noon for lunch, Elio (the name Dr. Schaechter preferred to be called by his friends and colleagues) had introduced into the noontime ritual a game called Botticelli, named after an Italian artist and described as follows by Wikipedia:

Botticelli *is a guessing game which requires the players to have a good knowledge of biographical details of famous people. The game has several variants, but the common theme is that one person or team thinks of a famous person, reveals their initial letter, and then answers yes/no questions to allow other players to guess the identity. The game takes its name from the famous person having to be at least as famous as Sandro Botticelli, who is also the answer to the archetypal question, "Did you paint a picture of Venus rising?" referring to his painting "The Birth of Venus."*

Given Elio's background, a newbie like me should not have been too surprised when first introduced to this lunchtime ritual. He was born—and lived up to his teens—in Milan, Italy, where he had acquired a generous exposure to fine art. Those games of Botticelli were always entertaining, often educational and occasionally hilarious.

Sharing a laboratory with Elio was a perpetual delight. He could speak at least six languages and could be heard singing operatic arias in one or another of them as he puttered around the lab. And he wasn't so tied to his lab bench that he was willing to let the rest of the world go by without occasionally taking time to investigate. So it was that one day he announced to everybody working in the lab, "Let's go to the tobacco auction." And so we did.

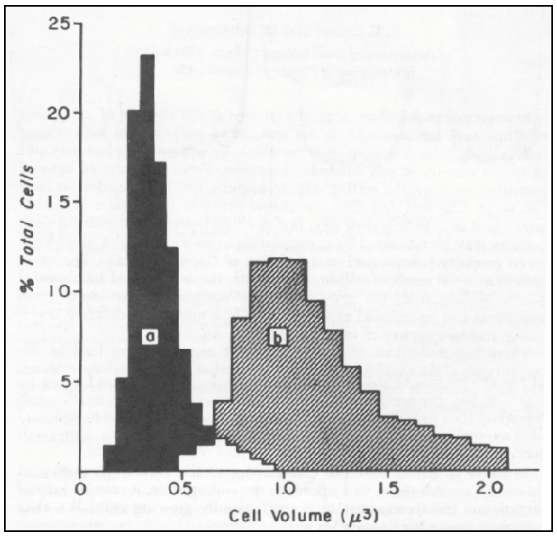
I grew up listening to Lucky Strike cigarette commercials on the radio. Those commercials always opened with the voice of a tobacco auctioneer named L. A. (Speed) Riggs, calling for bids in his melodic, sing-song babble. Now, for the first time, I was going to experience the context in which that babble played out in real life. We didn't have to drive far to find the warehouse in which the auction was taking place. There we found row after row of cured tobacco, arranged in piles of perhaps a few hundred pounds each. In the paths between the rows we found the auctioneer on one side and the tobacco company buyers on the other. Each pile was auctioned separately…and quickly, because the warehouse was full of them. I do recall being reminded of the voice of Speed Riggs on the radio as watched that auctioneer at work.

About six months after I arrived in Gainesville, a new faculty member was added to the department. Dr. John Cebra was an immunologist who came to the department after a postdoctoral tenure in Israel. We became close friends, and remained so until his death a few years ago. John (and his wife Ethel) were great nature lovers and inveterate bird-watchers. I never acquired his fascination with birds, but I did enjoy spending time with him exploring what northern Florida offered in the way of flora, fauna and natural resources. One of those resources was Juniper Springs in the Ocala National Forest and the stream that was the run-off from the spring. Near the origin of the spring a National Forest concession rented canoes for a seven-mile trip down Juniper Run—and provided a pick-up at the end of the run. So, sometime in that first year in Gainesville, our family joined Elio and his wife Barbara and John and Ethel

and their daughter Judy for a canoe trip down Juniper run. The picture below shows the other two canoes and their occupants after we had traversed the earlier, more swiftly moving and winding portion of the run. On the left are Elio and Barbara and our daughter Susan and on the right are John and Ethel with their daughter Judy and our son Mark. Eric was in the canoe with us.

In regard to my work in the laboratory, I need to emphasize the point I made earlier that it takes time to initiate and get moving on a new research effort. When I applied for the postdoctoral position at the University of Florida, I assumed that I would probably need two years to complete some credible—and publishable—research work. And, I'm sure that if circumstances had remained unchanged, I would have had no trouble obtaining funding for a second year. However, things changed. During that first year, Elio was offered—and accepted appointment as a professor in the medical school at Tufts University in Boston. I was offered the job as his replacement on the faculty of the department at Florida. He moved to Boston in the spring of 1962—and I inherited his laboratory.

So, my work in that laboratory went on without any significant interruption. One aspect of that work I have yet to describe. That aspect involved a device called a Coulter Counter—a rather ingenious machine that could count bacteria directly; one at a time. Not only that. It could measure the size of each cell as it was counted. For my work, I was not primarily interested in counting them. I wanted to know how big they were—and how that size changed as I grew them at different rates. My reasons should be pretty obvious. My working theory was that faster growing cells have more ribosomes and, because ribosomes are assumed to function at constant efficiency, the number of ribosomes in a cell should increase directly with the rate of growth. A corollary to that theory would require that faster growing cells should be proportionately larger to make room for the increased number of ribosomes. The Coulter Counter gave me the opportunity to test that corollary.



The graphic on the left shows two different size distributions of bacterial cultures grown at different rates. The distribution on the left (a) represents a culture grown in a chemostat at 0.13 generations per hour (or about 7 hours and 40 minutes per cell division). The distribution of the right (b) represents a batch culture grown at 2 generations per hour (or about 30 minutes per cell division). Ideally, if all individual cells divided at

exactly the same size—and at exactly the same time after the previous division—their size distributions would not be so spread out as they are in the graphic. Obviously they don't. However, it is easy to see from the graphic that the average size of cells growing more slowly are much smaller than those that are growing at faster rates, validating my corollary theory.

By the time Elio left, and I became an independent investigator, I was just beginning to get useful results from my research. In fact, it was more than a year and a half into that project before I had obtained any results that could be considered worthy of publication. Meanwhile, I had to establish a new direction for my own research, apply for financial support, retool the laboratory to begin that work and undertake the responsibilities of being a full-time faculty member.

At home, the house we were renting was being offered for sale and we had to either buy it or move. Although we were benefiting from the somewhat higher salary my faculty appointment provided, we were in no position to consider purchasing a house, so we had to find a new place to rent. We did. It was at the other end of town, but no farther from the medical school and still within walking distance of its neighborhood elementary school. It was on a large corner lot with an extensive back yard and best of all—at least as far as the kids were concerned—someone had left a good sized back yard pool in the vacant lot behind our house. The pool and its frequent occupants are shown above. The photos on the left show the expanse of that back yard area and some willing models adding personality to a couple of views of the front of the house.

A number of significant things happened as we settling into this house, not the least of which was the birth of our fourth child, Karen, in August of that year (1962). This was a three-bedroom house and the owner had converted the carport into an additional bedroom. So we had space to accommodate this addition to our family. The most revealing picture of Karen while we were living in this house was taken when she was old enough to scoot around in stroller. She became known as "The Petunia Eater," and here is a photo of her caught in the act.

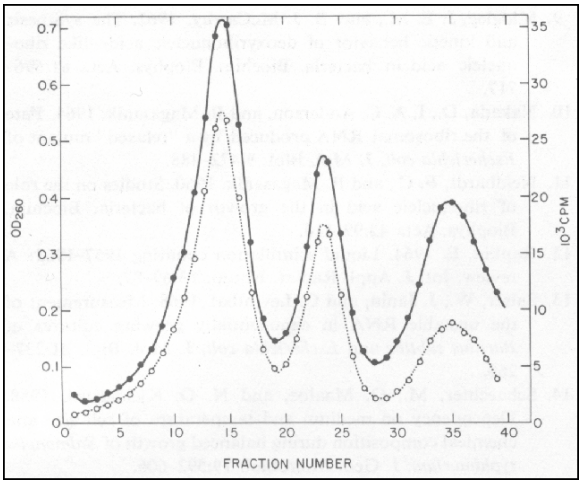
Without

question, the next most significant event in our lives while we lived in this house was the "Cuban Missile Crisis." As you might guess, people who lived in Florida at that time viewed the presence of those nearby missiles a lot more seriously than other Americans who lived more distant from the threat. We even had a missile-raid drill at the medical school. The civil defense authorities had determined that it was essential, in the event the state became the target of a missile attack, the faculty of the medical school would emerge from that attack capable of quickly reestablishing medical education in our part of the country. So, all faculty members and their families were asked to participate in a drill to assure our survival in case of an attack.

As it happened, the building housing the medical school was built in an area that had once been a swamp. You might ask how you can build a six-story building on a swamp. In fact, Archimedes figured the answer to that many years ago—you float it. As long as you have enough of the building below the surface, it will float. This building had (has) three sub basements. And so it was, late one afternoon as the Cuban Missile Crisis was developing, all of the faculty members and their families (in our case including a three-month-old baby) found their way to the third sub basement of the medical center to rehearse for the possibility of a disaster. Fortunately, the disaster never occurred.

Work in the laboratory continued to progress at a reasonable pace. However, things were piling up for me as I had to assume appointments to faculty committees, accept a position on the graduate faculty (including team teaching a graduate course with a professor from the Biochemistry Department), apply for a grant to support my research and move my operation to a new laboratory down the hall. So, when Elio and I received an invitation to give a paper at a symposium sponsored by the New York Academy of Sciences that fall (1962), I called Elio in Boston as asked if he could present the paper for me. I was the principal investigator of the work and should have been the presenter. He graciously consented. I was tremendously relieved and it became the first of three research papers from the short period of our work together.

Meanwhile, as I worked to complete the project I had started in Elio's lab, I began some new experiments with a high-speed centrifuge technique of a different kind. Centrifuge rotors had become available in which tubes of samples could be rotated at high speeds in containers that swung out on pins, so that the contents of the samples would sediment exactly in the direction of the centrifugal field. These chambers were appropriately called "swinging buckets."



If you then filled the tube with a sucrose (sugar) solution such that the concentration varied from high at the bottom of the tube to low at the top, a sample placed in a thin layer on the top could be separated into its different size components when the centrifuge was run for several hours in the cold. After that time, the plastic tube could be punctured at the bottom and its contents dripped out in fractions for analysis. The graphic on the left shows the results of an experiment with a sample similar to the one used in the analytical ultracentrifuge (page 5), except that, in this case, the direction of sedimentation is to the left—that is, the first samples collected were of the heavier components in the sample. This kind of sampling made it possible to do chemical, physical or radiological analysis of each fraction. (Note: I do not have any data such as these that were

published while I was in Gainesville, but this later experiment used the same technique as I described above).

As we entered our third year in Gainesville, we were required to move once again, as the owner of the house we were in wanted to put in on the market—and we were still in no position to consider buying it. This time, we only had to move a few blocks. The house was a bit smaller—and the yard was a lot smaller—but we adapted and went on with our lives.). Here are a couple of photos taken while we lived in that house.

One disagreeable thing about that yard introduced us to a local malady that took the boys on several occasions back to the emergency room at the medical center. The condition was called "cutaneous larva migrans" and was caused by the larva of hookworm from dogs. These larvae were abundant in yards where dogs had once been, and they attached themselves to the skin of kids that were playing there. Then they burrowed under the skin and began to migrate, leaving an inflamed, itchy track. To eliminate them, the larvae had to be killed (frozen) by being sprayed with ethyl chloride (the same stuff athletic trainers use to treat contact injuries in athletes.

Certainly, our most memorable event from the time we lived in that house was the assassination of President John Kennedy. I learned about the assassination from our department chairman as I went to the conference room for lunch that day. I don't remember what the class schedules were, but I think most of us took off early to watch events unfold on the television. I do recall vividly coming home from church that Sunday just in time to watch Jack Ruby shoot Lee Harvey Oswald live on national TV.

Sometime that school year (1963-64), I was assigned to be the faculty supervisor for the departmental "media kitchen." This facility was where all of the culture media were prepared for use in the teaching labs and in the hospital's diagnostic lab. It was a very active enterprise and employed a number of full-time people. I didn't have to have any direct supervisory responsibility in the facility because it had a full-time overseer, Mr. Brown, who managed its day-to-day operations. Given that, it should not have been a particularly demanding duty for me. Regrettably, that's not the way it turned out.

One day, early in 1964, Mr. Brown came to me with a complaint about one of his employees. The woman in question was chronically late, frequently absent and a perpetual source of conflict in the facility. I asked Mr. Brown what he thought should be done about it. He replied that he believed she should be fired. I told him that it was within his responsibility as her supervisor to do so and if he felt that was what should be done, he should do it. He did it and I thought the issue was resolved. It wasn't. The next morning I was called into the department

chairman's office and asked to explain what had happened. After I explained, he said, "You can't do that." He went on to point out that this woman was a single mother and would be put under hardship if she lost her job. I protested that my media kitchen was not a welfare agency, that I was charged with the responsibility making it work efficiently and that I could not do so without the freedom to manage the personnel efficiently. He said, "She stays!" I said, "I resign!" and I walked out of his office to draft a letter confirming my resignation from the faculty. In the letter I agreed to stay till the end of the school year.

Those last four months in Gainesville were a bit hectic, but I was able to wrap up my work, locate another job and move the family to new surroundings for a new adventure.