Like our own lives, the National Institutes of Health (NIH) in the late 1950s was an exciting work in progress. Transplanted from the Monod laboratory at the core of the *Escherichia coli* microbial genetics revolution in Paris, we tumbled unceremoniously into the NIH melting pot. There, we began a lifelong love affair with mammalian cells and genes and the mechanisms that regulate gene expression (1, 2). In this brief joint autobiography, we try to capture a little of the excitement of the time and the interactions among scientists whose influences we still treasure.

To bring things up to date, we also include a brief epilogue describing our current cell culture studies, which carry on directly from the culture optimization work we did at the NIH (albeit with a gap of fifty-plus years). Thus, we summarize our and other recent findings documenting the importance of culturing mammalian cells in incubators maintained at physiological oxygen levels (~5% O$_2$) rather than equilibrated with air (20% O$_2$). It is important to grow cells under these more physiological conditions to minimize oxidative damage and the accumulation of potentially deleterious mutations in all cultured cells. However, we emphasize here that this modern addition to cell culture technology is particularly important for stem and other types of cultured cells intended for therapeutic introduction into patients.

**Prologue**

*Lee—*In the early months of 1957, Len and I were in Paris, looking forward to several more years in our dream city, working with Jacques Monod (Fig. 1) (3) on the newly minted lacZ operon in *E. coli* (4, 5) and exploring diauxie (6) and lactose uptake in *Bacillus megatherium*. We had organized part-time childcare for our French-born toddler and were feeling altogether stable when a weather-beaten postcard arrived. It had apparently arrived at the California Institute of Technology sometime after Len had defended his thesis, been returned to the sender, been forwarded on to Len’s family address, and ultimately been sent on to Paris. By the time we got it, some four months had elapsed since the date on which it announced Len was to report immediately for induction into the United States Army!

Although Len had understood that he would be exempt from military service until he completed his fellowship abroad, his draft board had apparently decided otherwise and put him in to fill its quota for the year. There was no appeal at this point. Len was a fugitive, plain and simple.

Jacques calmed us down and came up with a great alternative. “This fellow Harry Eagle at the United States National Institutes of Health has been pressing me to spend some time in his laboratory. Why don’t you go in my place?” Truly a great idea: if Harry Eagle (7) was willing to organize a Public Health Service appointment for Len, Len could report for duty to the NIH instead of the United States Army. It would still mean a major upheaval in our lives, but it would

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1 Because the redox studies in our laboratory have largely been led by Dr. Kondala Atkuri, he will play a key role in the discussion of this work in the Epilogue.
immediately get Len out of trouble. He already had been thinking about working on somatic cell genetics with mammalian cells after we left Paris. What better place could he go to start this work (albeit a little sooner than he expected)? So, without further ado, letters were dispatched (phone calls were prohibitively expensive), permissions and appointments were received, and within a couple of months, we were on the boat, ready to start a new life and career growing mammalian cells at the NIH in Bethesda.

Well, things were not quite that simple. Len was on his way to a new life and career, “carrying a pipette for his country” at the NIH (instead of carrying a gun in the army). However, I was at loose ends. Although Jacques Monod welcomed me into his laboratory as Len’s co-worker, the NIH was not so welcoming for husband-wife teams, particularly when the husband was a new recruit and the wife did not even have a bachelor’s degree. I was basically cut adrift. Len, ever resourceful, solved the problem. As soon as we arrived at the NIH, he organized an interview for me for a laboratory technician job with Bruce Ames (8), whom he knew from Caltech times (9). Bruce had recently been appointed as an independent scientist in Dr. Herbert Tabor’s section of the then-National Institute for Arthritis and Metabolic Diseases (10). We hit it off well, and Bruce decided to take me on. Len and I were settled once again.

Harry Eagle and Mammalian Cell Studies

Len—In today’s world, with the knowledge and technical handling skills we have developed for sorting, characterizing, growing, transferring, and genetically modifying a multitude of normal and neoplastic mammalian cells, it is difficult to imagine a time when simply being able to grow these cells in culture and see them with a microscope was amazing. Harry Eagle (Fig. 2) (7, 11) had done just that!

Eagle and his groups had developed the culture media and conditions for routinely growing HeLa and other cells isolated from tumors. Others, notably Theodore (Ted) Puck (13), had certainly contributed to the field. However, Eagle brought it all together, developing the technology necessary for growing cells and formulating the mammalian cell culture media that to this day bear his name. He was awarded the President’s National Medal of Science for this work (11).

I arrived in Eagle’s laboratory when the basics were pretty much in place, and we could begin to think about how to introduce selectable markers so that we could model mammalian genetic studies on those conducted previously with bacteria, molds, and yeast (Figs. 3 and 4). Robert DeMars (14) had already joined the laboratory with the same goal in mind. Eagle put us into a large laboratory that we shared with another colleague whom I remember mainly because he was routinely contaminating his cultures by letting his tie brush against the neck of the open culture flasks he was feeding. (Yes, many of
us still wore ties when working at the bench, and, no, we
did not have culture hoods, so we routinely fed our cul-
tures by working with a Bunsen burner on an open table
in the laboratory. Don’t knock it; it worked!)

When I first arrived in Eagle’s laboratory, I pitched
into the general effort to improve culture media so that
routine work with different kinds of cells would be possi-
bile. I was particularly struck by the need to develop me-
dia and conditions to enable very sparse growth. We
needed conditions that would enable growing cells to
form visible colonies that could be picked and expanded
in new culture vessels, much the way microbial colonies
were routinely picked and used to isolate various types of
mutants.

The nutritional side of this work ultimately led to the
inclusion of serine and pyruvate in the medium (7). We
found that this was particularly important under condi-
tions in which the cells were sparsely distributed and
could not readily “crossfeed” each other with nutrients
that we realized were routinely available under more
crowded conditions. This was clearly important. How-
ever, in retrospect, the more nebulous side of the work
emerges as having a greater influence both on my life
and on mammalian cell studies as we currently know
them. Basically, the difficulties I encountered in seeing,
quantitating, and characterizing the cells I was growing
planted the seeds that ultimately drove me to develop
the fluorescence-activated cell sorter (FACS) (Figs. 3–7)
(2, 15–17).2

It would take several more years before the idea (and
the enabling technology) matured to the point where the
FACS could even crystallize as a concept (2). In the
meantime, I adapted my working mode in the Eagle lab-

oratory to longer experiment cycles (one week instead of
one day to grow a culture and test an idea) and got used
to squinting through a microscope and trying to make
judgments about cells growing on glass or plastic, very
frustrating for someone whose vision was (and still is) as
poor as mine. Overall, though, cells grew. We worked
out reasons for why they did not grow and ways to im-
prove growth. Progress was happening. I was enjoying
the challenge.

I have to admit, though, I was really unhappy about
doing studies with the HeLa cell line, which had been es-
tablished relatively recently (in comparison with today)
but already had been bottlenecked, rescued, passaged
through who knows what conditions, and perhaps worst
of all, isolated from what was (to me at the time) an alien
human tissue never to be seen again. Although HeLa was
the Eagle laboratory stalwart and had certainly done yeo-
man work in helping establish mammalian cell culture
conditions, I wanted a cell line that was close to a normal
accessible cell type: lymphocytes, in other words, and
more specifically, mouse lymphocytes.

This being my quest, it did not take long before I was
fortunate enough to establish a collaboration with Mi-
ichael (Mike) Potter (18), Clive Dawes, Lloyd Law, and
Robert (Bob) Roosa, six floors down and at the other end
of Building 10, the vast NIH laboratory building in which
the Eagle laboratory was housed. Mike Potter, then as
now, was a phenomenal source of information about
murine lymphocytes. In addition, he and colleagues had
succeeded in establishing and propagating a mouse cell
line derived from a methylcholanthrene-induced lymph-
oid tumor. The cell line, which they named P388, had
been put into culture within two years of the time I ar-
rived at the NIH (19–21). It was exactly what I was look-

2 This is work for which I (and, through me, my group) received several
honors, including the Kyoto Prize (2006).
agreed to share it with me. In fact, it became our laboratory’s mainstay cell line for many years thereafter.

Of course, nothing is ever as simple as it seems. P388 is still in use today, as is its macrophage counterpart, P388 D1. Although apparently lymphoid in origin, a “variant” crept in that had macrophage characteristics. At first, this was believed to be a macrophage contaminant that somehow got immortalized and was carried along with the lymphoid tumor. Then, it was ultimately believed to have been a macrophage all along. Mike Potter finally straightened this out by showing that both the lymphoid and the macrophage lines carry the same IgH chromosome rearrangements and hence are extremely likely to have originated from the same cell (20); but of course, we knew nothing about IgH rearrangements at that time. We did not even know that there was an immunoglobulin heavy chain!

FIGURE 4. Our family over the years. Upper, Jana, Len, and Lee at the Stanford University genetics picnic in 1961. Lower left, Berri in 1960. Lower middle, Lee, Berri (age 2), and Len after the great snowstorm of 1958 in Rockville, Maryland. Lower right, Berri and Jana in 1960.

FIGURE 5. Len and Lee in 1982 (left) and 1995 (right).
P388 turned out to be a convenient vehicle for the drug resistance studies that were central to thinking about \textit{in vitro} somatic cell genetics work at the time. The cells grew separately from one another without moving around too much after division, allowing us to identify (and try to “pick”) colonies/clones in sparse cultures. In addition, the cells were hardy enough to withstand the rigors of culture at the time, which (lacking CO$_2$ incubators) included having the media pH set by “gassing” the cultures by opening the stoppered culture flasks and blowing in a CO$_2$ stream for just the right amount of time. The methods were admittedly crude but nonetheless good enough to allow Bob Roosa and me to work out the need for pyruvate and serine in sparse cultures (22) and for Ray Bradley, Lloyd Law, Bob, and me to isolate a series of drug-resistant P388 lines that included “drug-marked” lines resistant to amethopterin, 8-azaguanine, or one of several fluorinated pyrimidines.

Overall, the connection with these colleagues and the new mammalian cell technology being worked out in Eagle’s laboratory were very exciting. Life at the NIH was good, too (except for the occasional snowstorm) (Fig. 4). Therefore, as I saw my two-year stint at the NIH coming to a close, I looked around for a way to stay on permanently. Within a short time, I got a very acceptable offer that included a good position and a good amount of space. By late November, I was poised to accept this NIH offer and to announce to our families that we would be staying in Bethesda for the foreseeable future.
Foresight, however, is not always that good. Just as Lee and I were starting off on a drive north for a Thanksgiving visit, I stopped off at the NIH to collect my mail. There, in an assuming airmail envelope, I found a very surprising letter from Joshua (Josh) Lederberg (23) in Wisconsin, offering me the possibility of being his first faculty appointee in the new department he was establishing at the Stanford University School of Medicine. I had met Josh initially in Wisconsin in 1954 when Lee and I stopped to visit his laboratory while driving from Pasadena to New York (Fig. 8). I talked briefly with him when he visited the Monod laboratory in Paris in 1956 and had a somewhat longish and quite interesting talk with him after a 1958 seminar he gave at the NIH. However, nothing in our discussions suggested that he was interested in anything more than finding out what I was thinking and doing in the laboratory. The job offer basically came out of the blue!

Lee and I were in a state of shock as we drove toward New York. She read Josh’s letter to me three times over. We knew that Stanford was restructuring its medical school, which had been relocated to the main university campus in Palo Alto to enable increased emphasis on basic science in the school. Arthur Kornberg had just moved his entire St. Louis department to Stanford, creating the prize-winning biochemistry department that endures today. Now, Josh was telling us that he was about to establish a genetics department that would operate side by side with the biochemistry department and that he wanted me to join this department.

Our first thought was “We can’t really do this.” We liked the NIH, we liked our colleagues, we had developed great relationships, and we had even bought a house. And our parents thought that even the NIH was too far away for them, with one grandchild to play with and another on the way. Besides, the years at Caltech in Pasadena left us with a distaste for smog and California in general.

However, opportunity wouldn’t stop knocking. As we thought about Stanford over Thanksgiving, Lee and I began to realize that Josh’s offer was too good to pass up. Finally, Harry Eagle clinched the decision. When we got back to Bethesda, he told me that he had recommended me to Josh and that, “quite frankly, this is the best job going this year!” What else could I do but accept?

The plan with Josh was for me to visit Stanford in December or early January to finalize the appointment. In those days, people did not fly back and forth for interviews and discussions. A bunch of letters, a costly telephone call, and the deal was done: I told my NIH colleagues that I would not accept their generous offer, and I made my reservations to fly to San Francisco. A hitch developed when Josh wrote to say that he would have to put off my visit until February because he had to do some traveling and would not be available until then. We found out later that the “traveling” was necessary to collect the Nobel prize (Fig. 9).

In any event, trip to Palo Alto aside, I still had roughly eight months to finish my work at the NIH and enjoy the interactions I had developed with my colleagues there. I
was quite excited about working with P388 and the other cell lines that Mike Potter, Bob Roosa, and I had developed (19–21). We completed one study with the lines before I left the NIH (24), and I continued to use them in my early work at Stanford (25, 26). They also proved useful for studies by others that focused on transferring genetic material between cells (then the rage in bacterial studies) and on elucidating the mechanisms responsible for drug resistance.

This work with P388 and with the colleagues with whom I worked at NIH wound up having a far greater impact on my scientific life than the individual findings would suggest. The collaborative work with this mouse cell line and with Mike Potter in particular introduced me to a fascinating world of mouse genetics. This was a time when inbred mouse strains had only recently been established and were explosively intersecting with tumor immunology and with the discovery of genetically controlled cell-surface antigens on normal and neoplastic cells.

While I was at the NIH, these interests mainly took the form of reading, seminars, and endless discussions with the people in Mike Potter’s and Lloyd Old’s laboratories. The more I learned, the more I became convinced that it could be possible to mutate these cell-surface antigens and use them as markers for genetic studies in culture. The problem was that, although the antigens were known to be under genetic control, no one knew what they were. No lesser a light than P.B. Medawar (27) opined that the major histocompatibility marker on mouse cells (the so-called H-2 antigen at the time) might be made of DNA, leading me some time later (when I arrived at Stanford) to isolate and characterize these antigen-bearing molecules and show that they were actually cell-surface glycoproteins (28, 29).

With this new interest burgeoning just as Lee and I were about to leave the NIH and start our Stanford careers, I managed to visit George Snell (30) at The Jackson Laboratory at Bar Harbor, Maine. George, a wonderful and learned New England gentleman, confirmed my instinct that H-2 and other mouse cell-surface antigens could be useful in vitro genetic markers. Thus, he set me (really us) on a lifelong course (2) in which we focused initially on mouse lymphocytes and immunoglobulins as models for human studies (25, 26) and ultimately, once we developed a function FACS and established the Milstein technology in our laboratory, on establishing several of the earliest hybridomas (31–39) and characterizing the genes, surface antigens, cells, and properties of the mouse and human immune systems.

On the whole, the years I spent in Eagle’s laboratory at the NIH were exciting, formative, and intellectually expansive. They were also highly productive, particularly in terms of the long-term influence my colleagues and I had on mammalian cell culture methods and the findings these methods enabled. However, as I look back, I realize that the people I met and the focus on mammalian biology, genetics, and human disease that they transmitted to me are really the enduring legacy of our NIH years. Seminars and conversations with Robert DeMars, Michael Potter, Robert Roosa, John Fahey, Lloyd Law, Harry Eagle, James Darnell (40), DeWitt Stetten, Jr. (41), Herbert (10) and Celia Tabor, Bruce Ames (8), Alan Mehler (42), William Dreyer (43), and Gordon Thompsons (44) stand out in my mind at the moment. (I am...
sure I am leaving out others who were equally important.)

We owe much to these scientific friendships, not least because they have been the source or the “next stop” for some of the wonderful students and fellows who have peopled our laboratory over the years. As we meet or talk with each other from time to time, I am continually aware of how valuable my NIH roots really are.

Lee—As usual, Len’s comment hits the nail right on the head. I was very lucky that Bruce Ames (8, 45, 46), one of Len’s Caltech colleagues, had moved to the NIH and secured a permanent position doing bacterial genetics and metabolic pathway studies. When we arrived in Bethesda, Bruce was looking for a research assistant. I applied, and he decided to hire me. Thus, while Len was diving into uncharted waters in the mammalian cell culture world, I continued to do bacterial work fairly similar to what I had been doing with Len in Jacques Monod’s laboratory.

I was delighted. I did not imagine that within a couple of years, I would move permanently into the mammalian world, and I certainly did not imagine that the Salmonella work I would do with Bruce would have direct consequences on my later mammalian work. In fact, I did not even realize the connection between the histidine biosynthesis regulatory studies I was doing with Bruce (46) and the immunoregulatory mechanism concepts that my group and I developed at Stanford years later (47). It took Caltech biophysicist Max Delbrück’s (Fig. 10) (48) unique mind to recognize the connection and show me how to put the jigsaw pieces of my life together. It all happened this way...

When Len and I arrived at the NIH, Bruce Ames had only recently started at the NIH, or at least he had only recently gotten approval for a technician (what we now usually call a life science research assistant). While Len was still at Caltech, I had worked for Marguerite Vogt (49) and Renato Dulbecco (50) as a kind of glorified dishwasher, and I had also worked as a technician/secretary for embryologist Albert Tyler (51). But, truth be told, I did not do much actual benchwork there, so most of my experience had been working with Len at the Pasteur Institute, where I did some benchwork but mainly did a lot of data collection (with manual instruments) and a great deal of computation and analysis. In any event, I guess I was bright and knowledgeable enough to convince Bruce to take me on and teach me what I needed to know to do the benchwork that he needed.

It was an interesting match. I was Bruce’s first technician, and he was my first boss. I think that I got the better part of the deal. Bruce was known at Caltech for his efficiency and organization in the laboratory, where his thesis was based on the extraordinarily rapid mapping of a biosynthetic pathway (9). He had only gotten better in the interim, and his laboratory was already in shape and productive before I arrived (52).

I understood what Bruce was doing and why (defining the enzymes in the histidine biosynthetic pathway in Salmonella). However, I had none of the discipline required to efficiently characterize the properties of each enzyme in order along the pathway. I would do the work he patiently laid out for me each day, and I would collect and record the data as required. However (at least it seems to me now), I was forever noticing some odd bit of data and pointing out how it might be followed up, rather than following Bruce’s lead and keeping strictly to the trajectory he set for me. The work got done, but I was certainly a sore trial for him.
I loved the work environment in Bruce’s laboratory, which was situated in one of the smaller (two- to three-story) buildings next to the mammoth Building 10. I knew everyone in the building, at least in passing (on the stairway, for example). Herb and Celia Tabor had laboratories at the end of the hall and always had a pleasant word for me. I think Alan Mehler (53), who taught the wonderful NIH graduate level enzymology course that I took, had a laboratory in our building (or perhaps his laboratories were in Building 10, and he was just a frequent denizen in our hallway, maybe on his way to and from the automatic Cary spectrophotometer that was housed on our floor). In any event, science conversations of all types were easy and frequent in the building. I was welcomed and treated like a younger colleague, much to my delight.

After I had been working with Bruce for a little less than a year, he decided to spend a couple of months with Arthur Kornberg (54) and his group at Washington University in Saint Louis (they moved to Stanford shortly thereafter). In today’s world, Bruce and I would not find this separation particularly disruptive. We would just keep in touch on a daily basis by phone, send data sheets out by computer, and maybe even use the Internet to get on Adobe Connect or Skype and look at data sheets together. However, back then, long-distance telephone calls cost $3 or more per minute. Only movie stars and politicians made calls that were not emergencies! Mail, what we call “snail mail” now, was the only communication medium, and even airmail was expensive, so Bruce and I had to carefully plan the work I would do and hope I would not run into problems that would require his immediate attention.

Good luck! Of course, a huge problem cropped up even before Bruce boarded the train to St. Louis. That morning, really just before leaving, Bruce completed the synthesis of histidinol, the substrate for the histidine biosynthetic enzyme I was to characterize in his absence. As he rushed out the door to a waiting taxi, he handed the synthesized product to me with instructions to take the spectrum immediately to check its purity. If it was okay, as we were sure it would be, I was to continue with the series of experiments he had outlined to define the properties of the enzyme that I was entrusted with characterizing.

I put the vial Bruce gave me in the fridge, listened to a few last-minute instructions, and waved him out the door with the proverbial “Don’t worry. Everything will be okay.” I then went to lunch, came back, drew a tiny aliquot from the vial, diluted it appropriately, and put it into the Cary (spectrophotometer) to check the spectrum. The roof caved in! The spectrum was completely wrong! It was not that what Bruce had synthesized was contaminated. The synthesis had simply failed!

Well, I was not completely certain that I had interpreted the spectrum correctly. (Actually, I was hoping I was wrong.) In any event, I went straight to Alan Mehler because I knew he knew the enzymes and substrates in the pathway I was working. As soon as I showed the spectrum to him, he confirmed that Bruce’s synthesis had not yielded the histidinol Bruce thought he had synthesized. I was completely devastated.

“What should I do now? Bruce thought the synthesis was okay. He didn’t leave me with any contingency plans. I have no idea how long it will take to get in touch with him. I don’t know what to do!”

Alan, always calm, suggested a simple plan: write to Bruce as soon as possible and then just find something useful to do until Bruce replied. I took a day to think before I wrote to Bruce, and in my note to him, I told him both what had happened and what I planned to do until I got other instructions from him.

The end result, at least as I remember it now, was that because this was the last enzyme in the pathway and we had completed all of the work that was needed for the earlier enzymes, Bruce decided not to send instructions for a new project. Instead, he said to carry on with the work I proposed, which centered on determining whether the expression of the enzymes in the histidine biosynthetic pathway was coordinately regulated in the same way that the expression of the enzymes in what became known as the lacZ operon was regulated, i.e. that limiting growth in the absence of histidine would result in the coordinate induction of the histidine biosynthetic enzymes. By the time Bruce got back, I had collected enough data to suggest that this was indeed the case.

Although Bruce was quite interested in these findings, he decided to complete the histidinol work first so that the full pathway was definitively described and worked on. Therefore, as soon as Bruce returned, he synthesized the compound we needed, and we went back into enzyme characterization mode. Several months later, Len and I realized that I was pregnant with our second child and that I would have to take a somewhat long leave of absence. Because Len’s two-year term at the NIH was near completion and we were already planning to move to California, this effectively meant that the time had come for me to terminate my position in Bruce’s labora-
tory. The histidine pathway work, in any event, was near-
ning completion, and Bruce was ready to move into a new
project.
I thought that this was the end of the story. However, I
was delighted to learn that Bruce decided sometime later
to focus on the histidine operon question that I had
opened while he was away, and I was even more de-
lighted to learn that he completed the work with Barbara
Garry and had included me as an author on the paper
that issued from his far more extensive and definitive
probing of this question (46).
Had Bruce not generously decided to include me as an
author on this paper, I am not sure that I would have
even remembered the work. However, I did not forget
the regulatory mechanism implied by both the lacZ
and histidine operons in bacteria (off when the end product
of the pathway is not needed and on full tilt when the
end product is needed). The bistable nature of this
mechanism sunk into my subconscious, only to bubble
to the surface later in response to Max Delbrück’s ques-
tioning. In essence, Max (48) recognized the similarity
between these bacterial growth regulation mechanisms
and the bistable model that my colleagues and I pro-
posed as a central regulatory mechanism operating to
control antibody responses in the mouse.
As many people know, Max developed multiple my-
eloma shortly before the end of his life. Interested in the
biology of the disease that afflicted him, Max asked his
Caltech immunologist colleague Ray Owen (Fig. 11) (55–
57) for some reading of interest. Ray (my professor and
lifelong mentor) was a bit at sea in trying to recommend
immunology reading to biophysicist and phage geneticist
Max Delbrück. Because immunology papers tend to be a
bit arcane (to say the least), Ray felt Max would do best
reading something that had a little more of the flavor and
generality of what we now call systems biology. The “im-
mune regulatory circuits model” paper that I had re-
cently published (47) fit the bill, so he put it on top of the
stack of papers that he handed over to Max.
A month or two later, Len and I came to Caltech to
join Ray in celebrating his fiftieth year on the Caltech
faculty. Max met us as we entered the patio housing
the opening reception. He surprised me by taking my
arm and steering me rather forcefully over to a quiet
corner.
“I read your circuits model paper. Tell me your influ-
ences,” he said. I must have looked at him blankly be-
cause he continued after a minute with “Who did you
study with? Where did the ideas come from?”
“Well, I learned immunology first from Ray, and then
from Av Mitchison (102), Gus Nossal (58), Klaus Rajew-
sky (59) and...”
“No, no, no,” Max cut in, impatient as only Max could
be. “No, no, where did these ideas come from?”
I was stymied. I had no idea what he was looking for, and apparently he had no way of telling me. The next day, however, he met me with an inch-thick green paperback. "Here, read this, and you will understand my question."

I took the volume and thumbed it. It was full of integrals and other alien equations that certainly did not look like they would have anything to do with me or immunology. "Max, I can't understand this," I told him. "You will. You will," he said, and summarily marched off.

I did not open the book until I was on the plane traveling back from Pasadena, and I suddenly realized Max was absolutely right. Not only could I understand what was in the book, but it actually had the answer to his question about my influences.

There were three sections to the "little green book," only two of which I can remember right now. The first section was about the lacZ operon, in which I was immersed when Len and I were in Monod's laboratory in Paris. The second was a description of the tryptophan operon, which was clearly similar to the histidine immunoregulatory circuit mechanisms (Fig. 12), the bacterial mechanisms Max pointed out were bistable systems akin to electronic "flip-flop" circuits. They operated either in the "on" or "off" position; they could not and did not operate for any length of time in the middle (61).

When I got back to Palo Alto, I immediately wrote Max a long letter, answering his question and thanking him for restoring me to my heritage. I explained my connection to the work discussed in the book and told him that I could now see where my ideas were actually grounded and hence how they traversed my history. Without my recognizing it, the NIH and Pasteur years had been a prelude to the immunoregulatory and systems biology work I would later do (1, 2, 27, 62–64, 70, 71). Max Delbrück’s query, initiated by the papers Ray Owen had given him to read, thus brought together the disconnected parts of my academic life and opened new perspectives that still influence much of our present thinking.

Len and Lee—These recollections of our formative years at the NIH would not be complete without a brief reference to the political side of our life in Bethesda and the greater Washington, D.C., area. At Caltech, just after we were married (Fig. 13), we had helped Matt Meselson (65) and George Streisinger (66) form a chapter of the Federation of American Scientists (FAS) (67), which at that time was heavily focused on the dangers that nuclear weapons and the fallout from nuclear weapons testing posed for society.

This was a time when prominent physicists, including Edward Teller (68), were arguing strongly that a little radiation in the atmosphere and fallout on Earth would not hurt anyone because there was a threshold below which radiation did not cause damage. Physicist and chemist Linus Pauling (69), then chairman of the Caltech Department of Chemistry, argued strongly against this view. Linus, of course, had the benefit of understanding the newly elucidated structure of DNA and its sensitivity to radiation and chemical mutagens (Fig. 14). Thus, he correctly argued that every increase in radiation, no matter how small, increases the probability of incurring harmful mutations in germ-line and somatic cells (70, 71). The FAS joined the debate, and we, as young biologists, joined the FAS.

We followed these issues from afar while at the Pasteur Institute. Jacques Monod (3), André Lwoff (72),...
François Jacob (73), and the other senior biologists, of
course, were in agreement concerning the potential for
genetic damage. (Seems funny, now, that this was even a
discussion.) However, the Pasteur Institute was just re-
covering from the German occupation of Paris and the
devastation of the war in Europe, so the political discus-
sions around the lunch table at Pasteur centered largely
around more local current issues (and answering ques-
tions about WWII, the Resistance, and other recent his-
tory). We all had a great visit and much political discus-
sion with Linus and Helen Pauling (one of my mentors)
but only briefly on the Pauling-Teller debate, in which
Pauling was so obviously correct.

When we arrived in Bethesda, we picked up the thread
of our earlier political work and renewed active contact
with the FAS. This soon led to our assuming some major
responsibilities in the Washington-based national FAS
office, where we played a key role in publishing the
monthly newsletter and did lots of other grunt work that
younger people often do.

Our “politics,” notably our aversion to exposing people
to low level radiation, also permeated our personal life
and resulted in a funny (in retrospect) battle with the
dental staff at the Walter Reed hospital. When Lee was
about five months pregnant with our second child, she
developed a severe toothache. We went for help to Wal-
ter Reed, which was the HMO to which we were as-
signed because Len was in the Public Health Service.
The Walter Reed dentists were highly competent and
had wonderfully modern equipment and procedures.
However, they were furious when we, notably Lee, who
was in the chair, refused to let them x-ray her teeth until
they draped her with a lead apron. They had never heard
that x-rays could cause genetic damage and told us we
were crazy for thinking the baby might be hurt.

We stood our ground. After an hour or so, with Lee’s
tooth aching all the time, someone dug up a dusty lead
apron, and the dentists (by then, the head dentist had
taken command) ostentatiously draped her with the
apron and finally did the x-ray. However, they made it
clear to us that they certainly did not intend to do this
for anyone else. We sometimes muse about how long it
took for them to learn modern radiation safety proce-
dures and whether they remembered the clash we had.
In any event, we are glad we stuck to our FAS teachings.

Perhaps the most memorable FAS event that we orga-
nized during our time in the Washington office was a
three-day workshop led by physicist/biologist Leó Szilár
d (74), who had been active along with Albert Einstein in
helping to establish the Los Alamos atomic bomb project
and who joined Einstein in opposing nuclear weapons
testing and their further development. Of course, we
were all called communists and anti-American, but, with
colleagues and leaders like this, who would care? Later,
at Stanford, we joined Stanford biochemistry professors David Hogness (75) and Dale Kaiser in forming a San Francisco Bay area FAS chapter and in going out into the community to teach about the dangers of fallout from nuclear weapons testing. This morphed into a full-scale effort to teach people that fallout shelters could not protect the population against atomic bomb attacks. This work, again ridiculous from a current perspective, was actually a necessary response to a campaign by the federal government intent upon convincing the population that such protection was feasible and could keep them safe.

Although the political work we did occupied very little of our time, we mention it here because it brought us into contact with many wonderful senior colleagues who were either actively or quietly supportive of our goals. These contacts, with whom we usually talked more about science than politics when we met, also became a lasting part of our NIH heritage.

**Epilogue**

*Len and Lee—*Curiously, or perhaps expectedly, our political work aimed at protecting the human population against increases in mutational load due to radioactive fallout made us highly sensitive to the need for decreasing people’s exposure to radiation and environmental mutagens. Even very low levels of such agents can cause permanent genetic damage and/or induce somatic mutations that could later result in lethal neoplastic growth. Thus, because many different types of cells are being cultured preparative to therapeutic transfer into human subjects, we were quite aware of how important it now is to protect cultured cells against mutagenesis while they are being cultured.

This led, recently, to our realization that the nearly universal current methods of growing cells in CO₂ incubators equilibrated with air (20% O₂) introduce medically significant sources of oxidative damage (and hence sources of mutation) into the cultures. Under these conditions, cells in culture are continuously exposed to incubator oxygen levels that are 2–8-fold higher than the oxygen levels that mammalian cells normally encounter in vivo (Fig. 15). Thus, the cultured cells are at significantly greater risk of accumulating mutations that could pose a serious long-term health risk in therapeutic transfers.

Fortunately, it is no longer necessary to risk incurring this damage. Modern laboratory incubators now readily enable culture of mammalian cells at oxygen levels comparable with those mammalian cells typically encounter in vivo (commonly 5% O₂). In fact, we and others have now shifted to routinely maintaining all of our cultures in these commercially available physiological oxygen (5% O₂) incubators.

Thus, almost unintentionally at first, we recently re-embarked on cell culture optimization studies that have now returned us, with pleasure, to our NIH roots and to the extension of Harry Eagle’s legacy. Dr. Kondala Atkuri, who joined our laboratory some years ago, has provided the principal leadership for this project. Therefore, we have asked him to join us in writing this epilogue.

*Kondala—*The success of hematopoietic cell transplantation in a variety of disease modalities has paved the way for the establishment of transplantation therapies in which the transferred cells are grown and differentiated in culture, rather than having been freshly isolated prior to transfer. Embryonic stem cell, induced pluripotent stem cell, and mesenchymal stem cell technologies now provide capabilities likely to make regenerative and personalized medicine a reality in the near future. Thus, because these therapies involve transplantation of cultured cells that will persist for decades in the recipient, every effort must be made to minimize the introduction of genetic damage that could result in future tumorigenesis and/or other health hazards.

Current cell culture methods have clearly provided a workable base for cell function studies, including those that have enabled development of regenerative and personalized medicine. In fact, much of our understanding of signal transduction, cell growth, and other basic biochemical and metabolic processes currently rests on data acquired with these culture methods. Similarly, much of the current stem cell technology has been elucidated with this technology.

However, science progresses by the recognition of its flaws. Thus, it is time now to reconsider and refine conclusions drawn from these studies, in which the cells were...
mainly cultured in incubators equilibrated with air (20% O₂) and hence exposed to oxygen levels well above their normal physiological range. Furthermore and perhaps most important from a medical standpoint, it is time now to recognize that using these old-style atmospheric oxygen incubators to culture cells destined for therapeutic use introduces tangible health risks for the cell recipients. Specifically, culturing cells at atmospheric oxygen levels increases genetic damage that ultimately results in neoplastic transformations.

*In vivo*, cells are exposed to oxygen levels that vary somewhat from body location to location but that are always substantially lower than the oxygen levels in air. Most cells in the body are exposed to oxygen levels that range from 2 to 12%, depending on their location (Fig. 15). When air is taken into the lung, the oxygen levels immediately drop to ~15%. They then drop further as the oxygenated arterial blood (oxyhemoglobin) is carried to other tissues in the body and drop further still as oxygen is released into the tissues and moves into venous blood. Thus, whereas arterial blood oxygen levels are usually 10–12%, the oxygen levels in venous blood are 5–6%. Hypoxia (Fig. 15) occurs mainly in tissues that have substantially decreased blood supply. For example, in the central mass of a non-vascularized tumor, tissue oxygen levels can drop to <1%.

At the tissue-capillary interface, an oxygen gradient is established: cells closer to the capillaries are exposed to oxygen levels of ~5%, whereas cells farther away from capillaries or in areas with low blood circulation may be exposed to oxygen levels that fall between 1 and 3% oxygen (76). Physiological oxygen levels for lymphocytes (76) and other cells in tissues that have a good blood supply normally range from 2 to 5% (reviewed in Refs. 77–82).

Incubator oxygen levels at this range (2–5%) match the oxygen levels most tissue-based cells encounter *in vivo*. Thus, these levels are most appropriate for maintaining cells in culture and are most appropriately referred to in discussion as “physiological” (Fig. 15). In our laboratory, where we work mostly with cells from lymphoid organs and peripheral blood, we routinely maintain our incubators at 5% O₂, which, on average, most closely approximates physiological levels for these cells *in vivo.*

Not surprisingly, science language usage being what it is, some investigators refer to the 20% O₂ levels in air as “normoxic” and to the range of oxygen levels inside the body as “hypoxic.” This usage implies that the oxygen levels to which cells in the body are exposed are hypoxic and hence below normal oxygen levels for cells *in situ*. As a corollary, it therefore implies that culturing cells at the normoxic (20%) oxygen levels in air is a good thing to do.

In addition, because hypoxic is used to refer to all oxygen levels in the body, this usage does not foster distinction between the healthy (physiological) range of oxygen levels (2–12%) in the body and the suboptimal (pathophysiological) oxygen levels (<2%) that occur in neoplasms and other oxygen-depleting pathologies. Thus, in our work, we refer to *in vivo* oxygen levels for cells in healthy tissues as “physiological” and to oxygen levels below 2% as “pathophysiological” (Fig. 15) (83).

These studies (84–96) responded to a clear and general need to enable mammalian cell culture under conditions that are more protective of animal cells and more suited to their natural physiology. The chronic oxidative stress induced by culturing cells at two to eight times the oxygen levels that they normally encounter *in vivo* results in changes in cell physiology, metabolism, and gene expression. These changes color our view of normal metabolic processes and our ability to grow healthy cells for therapeutic purposes.

Cells cultured for short periods (days to weeks) at atmospheric oxygen levels may or may not respond differently than cells cultured at physiological oxygen levels, depending on the response that is measured (84–96). However, the longer cells are cultured at atmospheric oxygen levels, the higher the chance that the cells will acquire deleterious genetic anomalies.

The metabolic and genetic changes introduced by growing cells at these high oxygen levels has important implications for all types of cell culture work. However, the genetic changes are particularly important for stem and other types of cells that are being cultured for therapeutic reintroduction in people. Although the therapy may be highly successful, the patients may later develop neoplasms or other diseases arising from genetic damage introduced into the therapeutic cells during the culture period.

Culturing cells for long periods at physiological oxygen levels that more closely maintain the *in vivo* cellular redox state (85) poses less of a risk. Cells cultured under these conditions show fewer mutations (97) and karyotypic abnormalities (98). Furthermore, they grow faster and maintain steady growth for a substantially higher number of passages, *i.e.* the cellular senescence that commonly occurs...
after 50 population passages for fibroblasts (the "Hayflick limit") is readily surpassed (80, 99, 100).

Importantly from a therapeutic point of view, stem cells and pluripotent cells have been shown to maintain their pluripotency significantly longer at physiological oxygen levels (79). Thus, whenever mammalian cells are cultured, but particularly when the cultured cells are destined for therapies based on re-injection into human subjects, it is both crucial and practical to maintain incubator oxygen levels that are comparable with those that the cells encounter in vivo.

Len and Lee—When we joined Harry Eagle's laboratory at the close of the 1950s, we knew that we were becoming part of a scientific tradition that would open new futures for biology and medicine. The modern cell culture “conditioning” studies that we have discussed above, which we initiated in our laboratory in 2002, continue this tradition and pay homage to Dr. Eagle and all of the other trailblazers who made this work possible. Now, as increasing numbers of laboratories recognize the importance of routinely culturing cells at physiological oxygen levels, the new technology will add to the impact of this early work on both the basic understanding of mammalian cell function and the exciting new uses for cultured cells in medicine.

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