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Essay

Ernest Everett Just Lecture, 1999*
The Value of Mentoring in the Career of a Young Scientist

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It is with humility and pride that I accept the E.E. Just award, and it is a privilege to present the Just Lecture for 1999. Just was a brilliant scientist in his time—rubbing shoulders with the geniuses of the time, including Loeb and Lillie, and walking in the shadows of Conklin and other developmental biologists. Just's tenets are today's paradigms for cell and developmental biology and the neurosciences.

Just has a special significance to me because he founded the Department of Zoology at Howard University. Looking back, Just would be proud to know that since its inception his department has graduated more than 5000 Bachelor of Science degree students. Just also developed the Masters program in Zoology at Howard University, the first at an HBCU. He also produced approximately 15 Masters students who went on to complete the doctorate degree, including Geraldine Pittman-Woods and Louis Hansborough. Just's department has produced more than 200 Master of Science degrees, and the department evolved to produce approximately 12% of the nation's African-American Ph.D.s in the biological sciences. Just indeed left massive footprints in the halls of academe—footprints that are difficult to fill but footprints and a career that are worthy of emulation.

I am an alumnus of Just's department where two University of Wisconsin–trained scientists served as my mentors: one was Charles Brown, a cell biologist; the other Harold Finley, an eminent protozoologist. They pushed and guided me toward a career in the biomedical sciences. They knew that at that time it was in my best interest not to get three degrees from the same institution, but that I should go away for further graduate studies.

Leaving Howard for Brown University represented a major change in my life. There I was confronted by a group of bright, well-prepared, and highly competitive graduate students. All 11 incoming students had the same schedule, interacting with each other and from two to four faculty in the same classroom daily for 1 year. Paul Weiss had just revised his textbook of biology, and we all had to assist in his undergraduate laboratories. We were privileged to be taught by brilliant developmental biologists like Mac V. Edds, Richard Goss, and then a young Paul Gross; cell biologists like J. Walter Wilson, Elizabeth Leduc, and Richard Ellis; and geneticists like Lederberg, Herman Chase, and Stanley Zimmering, and we had exposure to several bright young men and women like the Colemans, Gauthier, and Quevedo in the areas of cell and developmental biology.

Consider the national environment during this period—the ASCB was only 2 years old, and the founders, including Fawcett, Palade, Porter, and Swift, ruled supreme. We were all caught up in this national enthusiasm and as cell biologists considered ourselves "cytonauts"—exploring the cell—discovering and defining, rediscovering, and redefining the structure and functions of organelles. We benefited from two technical advances: the first was that the epoxy resins replaced the methacrylates, and aldehydes like glutaraldehyde replaced the formalin fixatives for electron microscopy. Everything was new—and surprising, and scientists like Ledbetter and Porter were defining the structure and functions of microtubules in plant and animal cells. We too presented information on microtrubule structure and function, showing their association with compensatory hypertrophy of kidney cells after unilateral nephrectomy, and described subplasmalemmal microtubules involved in motility of trypanosomes, which a year earlier appeared as solid filaments in methacrylate-embeded material, which we called subpellicular filaments. With Dick Ellis and Anne Weissman, a bright undergraduate student, we wrote a paper on microtubules involved in sequestering 6 of more than 50 mitochondria to form the middle piece of some invertebrate spermatozoa and described the manchette microtubules associated with nuclei of spermatids differentiating to form spermatozoa.

In 1962–1963 Lehninger too had just published his book on the mitochondrion, with little mention of mitochondrial DNA. At the same time, Ris, Piko, and Steinert and others were pioneering research on DNA in mitochondria; that same year we presented information on the greatest mitochondrial DNA repository, which was located in the kinetoplast of the trypanosome. We were busily developing techniques to better visualize DNA in intact mitochondria in germinal cells and corpus luteal cells and to later visualize DNA circles with Lloyd Matsumoto on cytochrome *c* spreads.

This trypanosome mitochondrial DNA paper was my first citation classic. From 1963 through 1966, even as a graduate student, I published 10 papers in the *Journal of Cell Biology*, *Experimental Cell Research*, *Zeitschrift fur Zellforschung*, and the leading journals of the time. This was only possible because of good mentoring. Indeed the environment at Brown at that time was stimulating, and the faculty in cell and developmental biology took every opportunity to propel students into mainstream research. My mentors provided the environment, the means, the encouragement, and

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the guidance. In Elizabeth Leduc's absence I presented lectures in cell biology to fellow graduate students; in Dick Ellis's absence his electron microscopy course was covered: the mentors provided the opportunity to develop skills as teacher as well as researcher. The faculty demonstrated all five main ingredients of good mentoring: opportunity, support, guidance, confidence, and example.

Lets us not be fooled: being a responsible mentor is no easy task. However, the responsible mentor should have a vision of seeing his or her students as future leaders in their areas of interest, believing in their abilities and helping them to realize their potentials; again, these are key ingredients in building confidence. Creating leaders takes relentless guidance, individual supervision, endless optimism, inexhaustible patience, and constant encouragement. The good mentor–advisor reminds the students of their strengths and permits the students to learn from their errors and even their failures. The student also has the freedom to disagree with the mentor, especially on research interpretation.

Toward the end of my graduate studies, I had an opportunity to postdoc with Stanley Bennett at University of North Carolina or Russell Barrnett at Yale. One of my mentors stepped forward once more and said, "Would it not be interesting to study in France with Jean Andre? You would be able to visit the Louvre and other interesting places." Now who could resist such a suggestion, and so Elizabeth Leduc and Dick Ellis used their contacts to provide me with a most fascinating chapter in my life. I spent 2 years at the University of Paris as a Cancer Society postdoctoral fellow, working alongside protozoology greats like Faure-Fremiet and cell biologists including Jean Andre, Rene Charet, and Barbara Stevens and his staff of bright young men and women. We had lunch as a lab, talking about research and life in general. I especially enjoyed the fellowship, good red wine, and *jambon* sandwiches with French bread. Andre was charming and brilliant and unselfishly found time to work with each young scientist. There we worked to develop techniques to better visualize mitochondrial DNA, DNA and RNA synthesis using autoradiography, and to localize enzymes and energy sources in germinal cells.

We were actually looking for DNA in centrioles when we accidentally used the silver proteinate technique to localize glycogen in and around centrioles, axonemes, and motochondria. In collaboration with Paul Personne and Jean Andre we determined the spatial arrangement and function of the unique and bizarre mitochondrion that formed the middle piece of the sperm of *Helix aspersa*. We used ultrastructural cytochemistry to dissect the various compartments of the mitochondrion and spermatozoan, showing a prominent glycogen compartment complete with glycogen synthetase, glyceraldehyde-3 phosphate dehydrogenase, and phosphorylase activity; lactate dehydrogenase and all the Kreb's cycle enzymes in a matrix compartment; and cytochrome *c* oxidase activity (electron transport chain) in the crystalline compartment that immediately surrounded the axoneme and the glycogen compartment. The axonome itself had prominent ATPase activity. Based on these findings, the following schema for middle piece–mitochondrial functioning was developed by Paul Personne and myself.

In Andre's lab we extensively exploited the diaminobenzidine reaction for the localization of cytochrome *c* oxidase activity by studying spermiogenesis and middle piece differentiation in different invertebrate species—and later fertilization in sea urchins and the fate of the sperm mitochondrion after entry into the egg.

The Paris experience provided lifetime links with several leading scientists of the time, and even as a postdoc I had access to brilliant scientists including Jean Brachet, Alberto Monroy, Baccio Baccetti, Bjorn Afzelius, then young Lisa Perotti, Paul Personne, and so many others. I published 15 papers from this laboratory, mostly with Paul Personne, and all this time in Paris I never visited the Louvre; that was a pity.

Returning back to the USA I had the opportunity to work directly for Don Fawcett and alongside some of the leading biologists, including Harold Amos, Sanford Palay, Betty Hay, Torsten Wiesel, Sus Ito, Jean Paul Revel, and so many young and dynamic postdoctoral students and instructors like Leak, Eddy, Trelstad, Hamilton, Coggeshall, Bolender, and Dym to name a few. We redefined the role of the manchette microtubule system in determining the shape and metamorphosis of the spermatid nucleus—perfected the technique along with Arnold Seligman to visualize cytochrome *c* oxidase activity in mitochondria and to later use this technique along with Lisa Perotti to determine respiratory quotients of muscle and pancreas.

At Harvard under the influence of colleagues and mentors like Morris Karnovsky we developed techniques to visualize tracers like myoglobin and cytochrome *c* to investigate vascular permeability in tissues. Indeed during the years we were at Harvard we were exposed to giants like Russ Barnett, Palade, Farquhar, Jamieson, Stanley Bennett, Arnold Seligman, Jake Hanker, and Alex Novikoff. At Harvard, it was possible to realize many of my aspirations in teaching and research, studying the distinctive and impressive styles and manners of Jean Paul Revel, Don Fawcett, Morris Karnovsky, and others. Over time, my mentors have become genuine colleagues, a true indication that the mentoring relationships have been successful. I learned from these individuals that good research was an important key to success in the academic profession; however, only as a responsible mentor can one ever become a good university professor. I believe that my career was launched at that time. As a point of information, it was under the Karnovsky–Jamieson ASCB presidencies that the Minority Affairs Committee (MAC) really gained recognition and was formalized. This was an important event, because from 1963 to 1973, only Everett Anderson, Lee Leak, and myself as African Americans were present at the annual ASCB meetings, and today, thanks, in part, to the MAC, especially through the efforts of former members, including Peter and Birgit Satir, John Browne, Langford, Wyche, Gonsalves, and others, the reconstituted MAC was able to increase the numbers of minorities at ASCB meetings.

Well, times changed. I had to grow up and find a real job, which I took at the University of Chicago's Pritzker School of Medicine. In fact, I inherited Bloom's lab. Finally I had my own lab, nice office and technicians, and graduate students. In addition, we had a progressive Cancer Center, Sickle Cell Center, and other research centers and a hot bed of modern cell biology research at the Whitman Laboratory. Next door to my lab were developmental biologists like Aaron Moscona, his wife, and then a young Don Fischman and young Shimada. Cell biologist and teacher supreme Hewson

Swift ran the Whitman, surrounded by bright postdocs, including Eugene Vigil. The fantastic Regenstein Library was 100 paces from my door. To my dismay, my responsibilities increased 100-fold, and I had to face 100–150 medical and graduate students daily. Anyway, I was fortunate to be awarded the first outstanding teacher award by the medical students, won the Anne Langer award for cancer research, produced my first two M.D./Ph.D. students, Dennis Slamon and Mike Press of herseptin fame.

The Cancer Center was run by Elwood Jensen and housed two-time Nobel Laureate Charlie Huggins and Guy Williams-Ashman, who were always available for discussions on breast cancer. While Elwood Jensen and Eugene DeSombre and others at the Ben May focused their attention on the estrogen receptor as a marker for hormone dependency, my students and I decided to search for more reliable marker proteins for estrogen dependency. Using the dimethylbenzanthracene-induced rat mammary tumor model, which is an estrogen-dependent tumor, and the the diaminobenzidine reaction, we identified an oxidoreductase, estrogen-induced peroxidase, as a prominent marker enzyme in these estrogen-dependent breast cancers. Estrogen withdrawal resulted in regression of the tumor and attendant loss of endogenous peroxidase activity; the addition of estrogen resulted in regrowth of the tumor and resynthesis of endogenous peroxidase. Actinomycin D and anti-estrogen administration blocked tumor growth. We proved that an endogenous peroxidase was a reliable biochemical marker for estrogen dependency in some rodent breast cancers.

During this period I also had an interest in reproductive biology and decided to collect cervical mucus samples from women during different phases of the ovulatory cycle. We found that the follicle-stimulating hormone–luteinizing hormone surge in midcycle coincided with high estrogen levels and was associated with, as we predicted, high cervical mucus peroxidase activity. Peroxidase activity declined with the fall of estrogen and rise of progesterone levels. Isoelectric focusing techniques performed later by Burnett and Ruchel supported the findings that cervical mucus peroxidase was prominent at midcycle, and its presence in cervical mucus was a reliable marker for ovulation in humans.

Next we used the immature or ovariectomized rat model to investigate growth of the uterine endometrium after treatment with estrogen, progesterone, and Tamoxifen aziridine. The estrogen-stimulated uterine epithelium synthesized massive amounts of estrogen-induced peroxidase, which was secreted into the uterine lumen. Isoelectric focusing studies revealed the presence of at least 15 isoenzymes that had peroxidase activity, with isoelectric points ranging from 3.5 to 6.5. On SDS-PAGE gels the major peroxidase protein had a molecular weight of 70 kDa. Kanan Balan is still trying to purify and determine the amino acid sequence of this peroxidase, which comigrates with other acidic proteins, including cathepsin isozymes. The peroxidase is released from the epithelium directly into the uterine lumen, where it probably performs a spermicidal or bactericidal role.

The uterine model allowed us to investigate further the possible mechanism of action of estrogen and Tamoxifen, based on studies using radiolabeled oligonucleotides, Northern and dot blots, and antibodies to products of the early genes by Western blots. Roger Ramsamy in our labs discovered that estrogen induced in the immature rat uteri the expression of early genes for Fos, Jun, and Myc (15–30 min), and within 15–45 min we observed expression of *ras*, epidermal growth factor (EGF), and EGF receptor (EGFr) genes. Stancel and others previously demonstrated binding of EGF to estrogen-stimulated uterine cells. Based on these findings we postulated the following hypothesis for the mechanism of action of estrogen: the activated estrogen–estrogen receptor in the nucleus acting as a transcription factor binds to the estrogen-response elements on DNA. *myc, fos,* and *jun* expression are immediate, and 30 min later EGF and EGFr activity are prominent. The EGFr is subsequently inserted into the cell membrane and associates with EGF in the external milieu. Ligand–receptor binding elicits the signal transduction pathway and MAP kinase cascade, leading to phosphorylation of Fos/Jun or Jun/Jun previously synthesized. AP1 now becomes the transcription factor that returns to the nucleus to turn on the proliferation genes. What we appear to have here is cross-talk between estrogen and a growth factor that may represent an important component in the mechanism of action of estrogen in normal and neoplastic tissues.

Similar studies were performed using Tamoxifen aziridine; this estrogen antagonist blocked expression of oncogenes except *jun* but also blocked expression of the EGFr gene and elicited early expression of genes for the insulin-like growth factors, transforming growth factor–binding proteins (TGFBs), and their receptors. Tamoxifen-treated cells did not proliferate and differentiated and hypertrophied 10-fold by 48 h. The uterine epithelium ceased proliferation by 48 h after treatment with estrogen, after which time we observed expression of genes for the insulin-like growth factors, TGFBs, and their receptors. We postulate that TGFB–TGFBr interaction and the signal transduction cascade are the signals for these cells to stop dividing, differentiate, and eventually undergo apoptosis.

Well, after more than 35 years in academic life, I approach the twilight of my own career. I am pleased to see that the mentoring cycle has repeated itself, and my own protégés have become mentors to their students. So I would like to challenge the young people in the audience to acquire the skills of good and responsible mentoring. It is a good feeling to wake up to *Good Morning America* to see your student being interviewed for making major advances in breast cancer research or in the biomedical sciences; so by influencing the lives of students you will receive rewards that money cannot buy. Congratulations to J.K. Haynes, Sandra, Donella, Vince, and all members of the Minority Affairs Committee and the ASCB for the great job that is being done on our behalf.