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OBITUARY

David L. Stocum (1939-2023)

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David L. Stocum, a scientist whose contributions to and impact on the field of regeneration and developmental biology are legendary, and likely more pervasive than many know, passed away on 21 April 2023. His illustrious career, exploring and characterizing the fundamentals of limb regeneration in salamanders, spanned nearly 60 years. Much of his work dissecting the tissue-level logic of regeneration established the framework for the molecular investigation of regeneration taking place today. His generous spirit as mentor and colleague, encyclopedic understanding of the literature, and enthusiasm for each new discovery and its place within the larger picture of scientific understanding distinguishes him as a giant in the history of regenerative biology. David's career path, the transformative role his teachers and mentors played along the way, and his own role in inspiring the next generation of researchers speaks strongly to the importance and power of basic education to society.

Left to right: David Stocum, Doug Melton, Thomas Poole, Ray Keller. Photo provided by Ray Keller.

David was born in Ypsilanti, Michigan, USA on 15 February 1939. As a first-generation student, guided by his high school German teacher, Joyce Gilbert, he attended her alma mater, Susquehanna University, sixty miles downriver from home. David described his college experiences as an intellectual awakening in which he became a passionate believer in the power of higher education. In his senior year, he took the Air Force examinations for pilot training and was selected; however, changes in his vision, along with expanded interests in zoology, redirected him to pursue his Master's degree at the University of Illinois in Urbana-Champaign. He earned his Master's degree under the mentorship of Dr Ray Watterson, a renowned classical embryologist legendary for his ability to write with one hand while drawing diagrams simultaneously with the other. Ray Watterson introduced David to his life's work in regeneration. David's passion for teaching was ignited with his first academic job, a one-year appointment at Iowa Wesleyan College in Mount Pleasant, Iowa, where he found that he not only enjoyed, but was also good at, teaching. Not surprisingly, David remained connected to some of his students from that transformative year who went on to make important discoveries in biology.

David entered graduate school at the University of Pennsylvania in Philadelphia in 1964 and, during his thesis work, published several influential papers demonstrating that blastemas self-organize to form identifiable limb structures in isolation from any influence of the original amputated limb. Under the mentorship of Professor Charles Wilde, he established conditions to culture dissociated *Ambystoma maculatum* limb blastema cells, demonstrating their autonomous ability to form both cartilage and muscle *in vitro* in the absence of stump. In a second manuscript, he showed that such blastemas form the appropriate limb elements even when grafted to tail fins. This work clarified decades of conflicting conclusions on whether blastemas contained intrinsic patterning information. Characteristically, David meticulously cited the previous literature and their potential caveats while finding beautiful means to address the question at hand. David summarized his conclusions in the following words: 'Within six months, I had hundreds of cases of blastema autografts that had self-organized into morphologically normal limb structures in the absence of differentiated limb tissues. In addition, grafts of undifferentiated fore limb and hind limb blastemas maintained their normal fate when exchanged for one another on their respective limb stumps. The bottom line was that even the earliest blastemas possessed all the information required to perform all the cellular divisions, movements and interactions required to restore the normal skeletal elements and muscles, in the correct relationships to one another, that the blastema would have formed in the presence of adjacent limb tissue. No induction by signals coming from those tissues was required.' (Stocum, autobiography unpublished). David's thesis work revealed fundamental new properties within the regenerating limb. His discoveries have inspired the research programs of many and influenced advancements within Developmental Biology for more than half a century.

As a newly minted PhD, David returned to the University of Illinois at Urbana-Champaign in 1968 as an Assistant Professor of Zoology. There, he founded his own research program studying salamander limb regeneration. David created a space for independent thought and investigation and leaves an impressive legacy of undergraduate, graduate and postdoctoral students. In the

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following statement he shared his philosophy of teaching and training: 'The big lesson that I learned here is that your students are a big part of your success and gift to the world. They develop skills and experience beyond your own that push the frontiers outwards. Some advisors want to dominate their students. This is not the way to go! Be satisfied with incubating them and proud of what they do thereafter!' (Stocum, autobiography unpublished).

David incorporated undergraduates into his research team long before it was considered wise or fashionable to do so, many of whom have gone on to transformative careers in science. One of his undergraduate students was Doug Melton (Harvard University) who stated how fortunate he was to have had David Stocum as his first mentor in science. In Doug's own words: 'Dave introduced me to the fun and challenge of experimental science. To this day, I have a strong memory of collecting Tubifex worms at the sanitation plant in Urbana, perspiring side by side with Dave and Ray Keller (a PhD student at that time), picking the worms off filthy rocks. While it seems absurd that suffering in summer heat on the top of a sanitation plant collecting worms could be fun, working with Dave made it so. He was enthralled with an important and fascinating problem, limb regeneration, and shared his enthusiasm and knowledge without limit. Working on limb regeneration as an undergraduate in his lab was a most fortunate and determinative event in my education. Dave, more than anyone else, convinced me I wanted to try to be a scientist. So, with gratitude and admiration, I mark his passing as a most influential teacher and generous person.' David's commitment to his graduate student's lives and well-being extended beyond the laboratory. Karen Crawford (former PhD student, now St. Mary's College of Maryland) remembers: 'We were his family and, in that regard, he officiated at marriages, taught more than one of us how to drive a stick shift and supported us through the challenging transitions that often come with graduate school. He celebrated with us, commiserated with us, and helped everyone around him to find a way forward from whatever challenges they were facing.' It is also important to recognize that David allowed his students to pursue new research directions within and beyond his area of expertise. Ray Keller (University of Virginia) fate mapped the early development of the *Xenopus* frog as a graduate student in David's laboratory, setting the stage for an extraordinary career studying morphogenetic movements during gastrulation in vertebrates. David aspired to and shared the philosophy for life expressed by Kent M. Keith in his book *Anyway, The Paradoxical Commandments: Finding Personal meaning in a Crazy World*. One of the paradoxical commandments that best exemplifies David is: 'The good you do today will be forgotten tomorrow. Do good anyway.' Throughout his life, David did good.

David's own work and his mentorship of his trainees led to an important decade of science. To investigate proximal-distal patterning of limb regeneration in the pre-transgenic era, David and his students used tritiated thymidine radiolabeled or triploid donor animals to distinguish donor from host tissues (Stocum, 1975; Pescitelli and Stocum, 1980). They showed that when distal blastemas are juxtaposed to more proximal stump tissue, the stump proceeded with intercalary regeneration to fill in the gap. This assay was used many years later with tissue-specific GFP-expressing transgenics to show the cell-type-specific nature of positional memory (Nacu et al., 2013). In cockroaches, limb position has segmental resolution, but David showed that, in salamanders, the information must also be graded within a segment leading to seamless regeneration – an aspect that is still not understood today.

To delve further into patterning David and his colleagues explored adhesive differences between proximal and distal blastemas by co-culturing pairs of unlabeled and radioactively labeled blastemal mesenchymal tissues in hanging drop cultures (Nardi and Stocum, 1984). Blastemas from more proximal regions of the limb were found to engulf more distal-level blastemas. In contrast, when blastemas from the same limb level were co-cultured, wrist with wrist for example, they did not demonstrate engulfment behaviors but fused at their borders in a straight line. These observations implicated a gradient of cell adhesivity dependent on the proximodistal origin of the blastema, and provided an assay that would be used decades later by Brockes and colleagues to identify PROD1 as a retinoic acid-regulated cell surface molecule mediating such adhesive differences (da Silva et al., 2002).

Not long afterwards, it was discovered that exogenous treatment with retinoids resulted in an entire limb regenerating from a hand blastema (Saxena and Niazi, 1977). One of the first scientists to observe this response, Dr Iqbal Niazi, visited David's lab and developed intraperitoneal injection methods to characterize the stage-specific dose-dependent response of retinoids on limb regeneration. To characterize these retinoic acid effects, a novel *in vivo* assay ultimately termed 'affinophoresis' was created. This showed that a blastema, when grafted onto a regenerating limb, 'finds' its appropriate proximal-distal level on the host regenerate. The powerful role of retinoic acid to convert hand blastemas to upper arm identity was also confirmed in the intercalation assay (Crawford and Stocum, 1988a,b).

Simultaneously, David and his students established the role of retinoic acid in not only specifying proximal identity but also in conferring posterior identity to blastema cells (Stocum and Thoms, 1984; Kim and Stocum, 1986a,b). Engineered anterior half-only limbs, which normally do not regenerate, regenerated whole limbs from wrist level amputations when treated with retinoic acid, whereas posterior half limbs failed to regenerate at all. These observations were used later by McCusker and colleagues to define the molecular conditions required to generate accessory limbs (Vieira et al., 2019). Altogether, these foundational studies, performed between 1978 and 1987, largely by creating opportunities for blastemal tissues to reveal their identities through their cell behaviors and regenerative patterns, set the stage for many of our current analyses of regeneration and blastema interactions at the molecular level.

After 20 years at the University of Illinois, David was invited to become the Dean of Science at the Indiana University-Purdue University in Indianapolis (IUPUI). From 1989 to 2004 he served as an administrator facilitating tremendous growth and advancements of that urban university. Under his mentorship, his faculty competed successfully for government and privately funded research initiatives, expanding their research programs and scientific contributions. He created opportunities for students as well as staff members, empowering those around him through his confidence in their potential to excel. He exemplified equity, inclusion and diversity before it was something new, and lived this ideal as an important constant for genuine success. Despite the demands of leadership, David remained immersed in the literature and advancements in regenerative biology and its medical applications. His encyclopedic knowledge base in his field, coupled with his ability to craft clear, logical and concise manuscripts, led him to fill a void by writing a much-needed textbook for advanced undergraduates and graduate students entitled *Regenerative Biology and Medicine* (Stocum, 2006). Currently published in its second edition, this text has become a resource for anyone interested in contributing to this rapidly growing field of research. David ensured that all areas of

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regeneration were represented. One of us (K.D.R.-T.) particularly remembers: 'Dave contacted me in my early tenured career and asked me: "…please keep me on your mailing list for reprints. I'm in the process of finishing my book on regenerative biology and medicine and I have relied heavily on the papers by you and Takis on lens and neural retina regeneration".'

David shaped the careers of many in the field of regeneration outside of his scientific family. Dr Prayag Murawala (MDI Biological Laboratory) shares what so many of us can identify with. 'As a young postdoc, just like everyone else, I was influenced by his book *Regenerative Biology and Medicine*. We connected via email almost 10 years ago and our bond grew with time until his last days. I have rarely seen such inquisitiveness and depth of knowledge. In every exchange he was like a young student, inquiring about my experiment. However, the beauty of these exchanges was, he would always come up with some old literature that would help me refine my ongoing experiments. His encouragement has always pushed me to strive for better both as a postdoc and as a faculty. He remained an influential figure in my career and I will always regret not meeting him in person.' Kate McCusker (University of Massachusetts Boston) says: 'Every now and again we would email back and forth after I published something. Given the pioneering role he played in understanding the mechanisms of regenerative patterning, I was always a little star struck when I received a message from him! It is really a testament to how connected he remained to our community through all these years, even to us newbies.' His encouragement and kindness spread at all stages of one's scientific career.

David's passion for discovery led him to return to the research laboratory as a faculty member at IUPUI in 2004. Once again, his primary focus was on salamander limb regeneration and training the next generation of students. Undaunted by the advancements in molecular biology, his international research team explored regeneration at the level of gene and protein expression (Rao et al., 2009; Jhamb et al., 2011). His curiosity and enthusiasm for basic research persisted throughout his career. In addition, he became a member of the Regenerative Biology and Tissue Engineering Theme at the Carl R. Woese Institute for Genomic Biology at the University of Illinois in Urbana.

David passed away at Life's Journey Hospice in Avon, Indiana not far from his home in Indianapolis. He is survived by his wife Laura, son Richard Stocum and grandson Trent Stocum, Indianapolis, and son Derek Stocum of Champaign, Illinois. The community of regeneration colleagues are deeply indebted to his meticulous, foundational contributions that inspired and enabled the transition of the field toward molecular studies. He was a gentle authority of regeneration – his profound knowledge, kind generosity and encouragement guided us all.

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