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How Serendipity Shaped a Life

An interview with John W. Saunders, Jr.

JOHN F. FALLON*

Department of Anatomy, University of Wisconsin-Madison, USA

I interviewed John Saunders, in his beautiful home in Waquoit, Massachusetts, Cape Cod on March 10, 2001. John wanted to talk about the events in his early life that helped to shape his career. He noted that because of a failed construction bid, he chose to study science. Probably few people know that an off hand comment by B.H. Willier about the apical ectodermal ridge changed John's Ph.D. thesis research to his pioneering analysis of ridge function. Since his retirement in 1984, John has maintained a vigorous schedule. He continues to interact with students as a faculty member in the Embryology Course at the Marine Biological Laboratory in Woods Hole. He attends national and international meetings and more often that not is a keynote speaker. Recently, he was awarded the prestigious Conklin Award by the American Society of Developmental Biology. His high enthusiasm for the study of embryonic development is no less than the day I first met him in 1960. In our talk, it is apparent that he is delighted by the way in which molecular approaches to developmental biology have provided mechanistic insights into experiments he carried out during his long career.

John, what attracted you to science?

I was not especially "attracted" to science initially. I just "happened" on to it. As a lad in high school I found that elementary experiments in physics and chemistry held some fascination. A natural curiosity and a certain amount of physical dexterity enabled me to perform simple experiments easily. I was not, however, particularly challenged intellectually at that time. Only during my junior year in college did I give serious thought to a career in biological science.

That was at the University of Oklahoma?

Yes, but how I came to choose biological science must be approached from the standpoint of my childhood background. This background was one of relative poverty, but from this poverty arose a peculiar set of circumstances that brought me to the University. You have to realize that I was a child of the Great Depression of the 1930's. My childhood was spent in Oklahoma during the time when great dust storms impoverished the central United States. Poverty and hunger struck heavily in Oklahoma as in other parts of the Great Plains. People suffered from hunger and jobs were few. Fortunate, indeed, was the breadwinner who could earn as much as $100 per month during my childhood. There were times when we did not know if we would have something to eat. To have a job that paid any kind of a wage was something much sought after; to get a job was something that took preparation. To this end, my parents insisted on my getting an education; but they really didn't know why, except that it would help me to get a steady job of some kind, perhaps reading meters for the Muskogee (Oklahoma) Gas and Electric Company, or something of that sort.

Abbreviations used in this paper: AER, apical ectodermal ridge; EZ, Journal of Experimental Zoology; MBL, Marine Biology Laboratory; PNZ, posterior necrotic zone; ZPA, zone of polarizing activity.

***Address for reprints:** Dr. John F. Fallon. Harland Winfield Mossman Professor, Department of Anatomy, University of Wisconsin-Madison, Madison, WI, USA. Fax: +1-608-262-2327. e-mail: jffallon@facstaff.wisc.edu

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What were your parents' backgrounds?

My father was a barber. He went to the Oklahoma Territory in 1907. He brought my mother, his bride, there after the Territory became a State. Mother's education stopped at the Second Grade; my father was educated through the Seventh Grade. They both felt I needed to get an education. Certainly from my mother's standpoint, it was so I could make a living.

I began earning money at the age of eight by caddying on a golf course. The money I earned went to my mother to help feed the family. At the age of 13, my first year in high school, I started working at a little restaurant and was paid 50 cents a day, working from 4:00 in the afternoon until 10:00 at night, six days a week. I'd come in and clean the place up on Sunday. So my total pay was $3.50 a week, and this money went to the family.

And you worked like this while you were going to high school?

Yes. It was a very small school; there were only six people in my graduating class. The courses were not very challenging; I had a 98-point something average, which I achieved with no difficulty at all despite my working.

Graduation did present a challenge. This was 1936. There were essentially no jobs. The depression in Oklahoma lasted a long time, and in my hometown of Muskogee there was simply no work. My mother, however, had a tremendous drive for me to further my education. She somehow learned that there was a Federal Program in which the local Junior College participated. Under this program students could be hired to do various tasks for the benefit of the school and could earn twenty cents for each hour worked. So we went to see the Dean of the junior college, Miss Bessie M. Huff, whom we persuaded to enroll me in Muskogee Junior College and to put me on the Fedreal Program´s payroll. In junior college the courses were very limited. The main emphasis was on English, since the Dean was an English major. She taught two courses in English that involved a very rigorous review of English grammar, intensive training in expository writing and a heavy emphasis on classic English literature. Public speaking was a major part of my experience in English literature. For example, in my sophomore year I was expected to present to the class an analysis of an epic poem, a lyric poem, and a Shakespearean play. The quality of the analysis as well as the clarity of presentation was emphasized. I am much indebted to Miss Huff for her training in these matters, which contributed much to my later success in writing and speaking.

During both my first and second years I also studied French. My doing so led to a series of events that led to my becoming a student at the University of Oklahoma - something that seemed completely out of reach at the time - and subsequently to my career in Biology. This is how it happened.

A fellow student in the course was the son of a senator in the Oklahoma legislature. The young man was failing in his course because he was unable to pass a required examination on French irregular verbs. He hired me to tutor him, and we would get together, either at his house or mine, for regular tutoring sessions. At his house one night, during a tutoring session, the Senator came in. By way of conversation after introductions, he asked if I were going on to college. I said I had no plans, for I had no money. He asked if I would like to attend the University of Oklahoma and, of course, I said yes. He said if I saw to it that his son passed his French course, he would see that I had a chance to go to the University of Oklahoma. *"I´ll get you a job at the State Hospital in Norman during the school year and a job with the State Highway department in the Summer."* Well, I saw to it that he passed! I told the dean of my opportunity, and I suspect that she brought some pressure on the French teacher. Miss Huff wanted very much for me to go on to the University. So I went to the University of Oklahoma in the Fall of 1936, without a clue as to what I would do when I got there besides working at the Hospital.

So how did you go on from here to choose a career in science?

Having worked summers as a lifeguard at the municipal swimming pool in Muskogee, I came in contact with a sanitary engineer; one of whose duties was to check water quality. I had some vague idea that possibly I might qualify myself to do something of this sort, but I had no notion as to how I should go about it.

So I went to the University of Oklahoma with forty dollars in my pocket and the promise of room and board at the State Hospital. I was assigned as advisee of Professor Audie Richards, Chairman of the Department of Zoology, and a former doctoral student of Professor E.G. Conklin, of Princeton. Dr. Richards looked at what I had done in Junior College and saw that I was prepared for practically nothing. He signed me up for a Zoology Major. Because of deficiencies in my earlier education, I found it necessary to take 19 credit hours each semester of my remaining undergraduate years at Oklahoma University in order to complete a major in Zoology with a minor in Botany. I decided that biological science was really to be the foundation of my career when I got into an elementary zoology lab and, for the first time, saw living things under the microscope. I looked at ciliates and amoebae; I went out in the field and collected specimens. I thought that protoplasm was wonderful stuff, and I wanted to work with living things. But, I had no idea what I would do with a major in Zoology when I graduated. Recall, this was still in the 30's, still in depression times. Ph.D.s were going without jobs. At Oklahoma University, Ph.D.s were doing menial jobs in the stockroom or working as technicians in the Zoology Department.

Happily, working at the State Hospital allowed me to study. I took care of mentally disturbed patients working from 10:00 at night until 2:00 in the morning, which gave me four hours for study unless there was trouble on my ward. Any college student who studies four hours every night should be able to earn good grades. Naturally, I wound up with good grades, Phi Beta Kappa (academic honor society), and all that. So I told my advisor, Dr. Richards, I wanted to go to graduate school and get a Masters Degree.

I stayed in the Zoology Department and started my Masters' Degree in 1940. My Masters thesis was entitled *"Aberrant Mitosis in the Amnion of the Guinea Pig."* I am still fascinated by the fact that when daughter chromosomes pull apart in anaphase in the squamous cells of the amnion, they reorient themselves so as to form flat plates parallel to the plane of the amniotic surface. I finished my Masters degree and applied to graduate school at Duke, Hopkins, and Harvard. I was accepted and awarded stipends by all three institutions but Hopkins offered me the most money. Remember, money was still very important. During the second semester of my senior year in college, for example, I quit my job at the state hospital in order to spend more time studying mitotic figures in a small laboratory that was assigned to me. I had 25 cents a day to spend for food and slept on the floor in my laboratory.

I went to Hopkins with the idea that I would study the physiology and cytology of protozoa. When I got to Hopkins in 1941, the department head Dr. Benjamin Willier said, "I don't think you are ready to do that sort of thing; here is what we do" and handed me a stack of reprints. He told me to read them and if I had any idea of what I wanted to do to come back and talk to him about it.

So that was Willier's style, a graduate student thought up a problem for the thesis work?
Yes, it was.

What was it like to study in the Willier lab?
There were no formal courses; the only courses were seminars. You were expected to attend and participate actively in seminars on several topics that were considered most important at the time, particularly as related to developmental biology: all aspects of tissue interactions in development were emphasized; cytogenetics and population genetics were stressed (these were the big things in genetics when I first arrived at Hopkins); you were expected to be up to date in developmental neurobiology and in the endocrinology of development. Emphasis was on the experimental basis for major concepts in these fields. Professor Willier selected the most pertinent papers on which individual students were to report. You might be assigned, for example, the papers of Lewis Stone and others on the question of lens regeneration in urodeles. You were expected to undertake a critical analysis of these papers, emphasizing the pertinence and validity of the reported findings, and report to the assembled members of the seminar group. This was excellent professional training. Our presentations were rigorously criticized by Willier and by fellow students. Dr. Willier emphasized that to show your command of a topic, you should be able to convey the essence of your information in a chalk talk, reducing the essence of your presentation to line diagrams —to a few sentences.

How did this work in my development as a student investigator? It gave me a background comprising critical knowledge of the major principles of biology, especially as elaborated in Paul Weiss' classic work, *"Principles of Development"* – for example, the concepts of the morphogenetic field; the progressiveness of the organization of fields and of organ-forming rudiments; the principle of dependent differentiation, with subordinate concepts of embryonic induction; and the notion of inductive competence and response capacity, and so on all come to mind.

Against this background I began to look at earlier work of Willier and Rawles (Mary E. Rawles) on feather development in the chick embryo. Their work led me to the question of how feathers become organized into tracts. Presumably each tract is some sort of a subordinate field, and its properties must be progressively organized. The shoulder tract of the chick embryo appeared to be a very nice thing to examine. And I began preliminary experiments involving microsurgical manipulations of the tissues of the future tract.

Fig. 1. John Saunders, 1942 at the Hopkins University, *learning to do chick operations under the tutelage of Mary Rawles in the background.*

So you were doing this on your own?
Oh, absolutely. I went to Willier only when I could show that I had a plan of experimentation that could produce meaningful results. Before this I made simple microsurgical operations on the future tract and was able to show that I could simply reorient portions of the future tract and gain meaningful information about its organization.

So I then went to Willier who approved my idea, and gave me permission to draw on the stockroom for supplies and to enlist the interest of Mary Rawles to aid me in refining my techniques.

At that point, you had permission to do bench work?
Yes, but I was only just getting started when my work was put on hold during three years of service as an officer in the US Navy.

You saw combat during that time?
Yes, I served in a fairly safe berth as a member of ship's company on various flagships of the amphibious forces in the Pacific. We participated in nine island-hopping invasions. My ship finally wound up as Flag of the Commander of the Seventh Fleet in Shanghai, China at the end of the war.

When was all this?
From 1943-1946. I returned to Hopkins in January of 1946. One day Willier came into the lab and asked to observe the operation that I was doing. I stained the limb bud of a 3-day chick embryo with Nile blue, and illustrated my technique of reorienting a portion

of the prospective shoulder feather tract *in ovo.* In the course of my demonstration, Willier's attention was drawn to a heavily stained ectodermal structure at the apex of the wing, and he asked what it was. All I knew was that it was an epithelial thickening. I set out to learn more about it.

To do this, I began experiments to determine effects of removing the ectodermal thickening, which we subsequently called the Apical Ectodermal Ridge, or AER. Removal of the ridge resulted in truncation of the resulting limb at proximodistal levels depending on the developmental stage of the embryo at the stage of operation. This was exciting, but I did not take my results to Willier until I had carried out all possible controls: effects of exposing the embryo to air for prolonged periods; possible selective toxicity of Nile Blue; deleterious effects of heat of the lamp used to illuminate the operative field; effects of damage to the mesoderm and exposure to amniotic fluid. With all controls accomplished, I went to Willier with the results. He was very interested and encouraged me to continue these experiments as a basis of my doctoral thesis, which I defended in the Spring of 1948.

That was very rapid progress, since you arrived in 1946 and defended your thesis in May of 1948; the experiments were done very quickly.

Yes, they were done quickly but they were easy once the operative technique was mastered. I spent 9 months writing my thesis. This period was interspersed with a few experiments to clean up odds and ends. Writing a thesis under Willier's direction was the best training in the world for me. The department would not accept a thesis until it was ready for publication, at that time a very rigorous criterion. I would bring a draft to the chief, that is what Willier was called, and he would go over it, make suggestions and send me off to rewrite. We went through several drafts together until finally Willier said he couldn't think of any way to make further improvements. Then he sent me with my thesis to Prof. Bentley Glass, Editor of the Quarterly Review of Biology. Dr. Glass went over the manuscript line by line, questioned every phrase, every comma, and so on. When we could think of no further way to improve the quality of the writing, I sent the manuscript to The Journal of Experimental Zoology (JEZ).

Fig. 2. John Saunders (1945) *on the bridge of USS Rocky Mounts Flagship, 87th Fleet, in Shanghai.*

At the time, was JEZ the place to publish an embryology paper?

Yes, embryologists mostly published in JEZ, but also in the Anatomical Record or the Journal of Morphology; those were the top three. The manuscript came back rather quickly without so much as a change in punctuation. Thanks to Willier's training I had the same experience with some subsequent manuscripts later.

Obviously, the style that Willier had developed produced a significant number of leaders in the field over the next 25 years (e.g., Spratt, Hamilton, Trinkaus, Ebert and Konigsberg to name a few). How would you compare graduate education when you started with graduate education today?

As I observe graduate education today, I am impressed that it consists to a great extent in the mastery of techniques. Graduate students learn techniques that enable them to contribute to a principal investigator's goals. So, the aims of the mentor's grant determines to a great deal what the student learns.

Right now graduate students would take courses that would point toward a preliminary exam which permits them to go on and make a thesis proposal which would be equivalent to Willier saying you can go to the stockroom. The preparation that points to a preliminary exam doesn't necessarily produce the scholarly background that Willier approached in seminars.

Willier's seminars did lead to a written preliminary exam. The questions were broad and required the student to integrate vast fields of knowledge into a comprehensive reply. One examination question that I remember particularly was *"Cytology is the Handmaiden of Genetics. Evaluate this statement in the light of current knowledge in the fields of genetics and cytology."* We had four hours in which to plan and write the answer.

Do you think the preparations of students today prepares them for a full career?

I don't think I can speak about that with much authority since I have been away from active work too long.

Well, a significant number of students today go into the biotechnology industry. If they are technically competent, regardless of their scholarly background, they can get a relatively high paying job. In part, they may have self-selected and decided to go into a technical approach for their career.

That's where the money is. I suppose a student with my background might have jumped at a similar chance.

That leads us to an interesting question of whether you would be a graduate student today?
I don't know. It goes back to this background of poverty. And I could easily have gone a different way entirely. For example, the Summer before I began graduate study at Oklahoma University, I worked on a construction crew as a manual laborer. My foreman was an employee of a large construction company with offices in both Oklahoma and New York City. Over the course of that Summer, I impressed him with my capacity for hard work. War loomed at this time, and there were calls for bids on some of the locks on the Panama Canal. My company bid on one of the locks. If their bid had been successful, my foreman would have taken me with him to Panama and I probably would have remained in the construction trade, for I found it very exciting. Happily, as it turned out, I returned to school.

I agree, it was good fortune. I still wonder, if you did decide to go to graduate school today, what do you think would attract you?
I really don't know what I would do today. I fell into almost everything. My training at Hopkins prepared me to take advantage of opportunities - serendipity!

You graduated from Hopkins in 1948 and went to do a postdoc with Paul Weiss at the University of Chicago.
It wasn't exactly a postdoc. My first public presentation of the apical ridge work was at a meeting of the Society of Zoology in Chicago. Weiss and Willier jointly chaired my session. After I gave my presentation, Weiss stood up and made the comment that this was the most significant piece of research that had appeared in the field of experimental embryology in the last 50 years. That, of course, immediately led me to all kinds of job offers. But Weiss himself offered me a job and I quickly took him up on his offer.

I was very naive, not very smart. I thought teaching was something I could do. So when Weiss said do you want to come to my lab, I asked in what capacity. He said I could work on his grant, but I said I wanted a teaching position that allowed plenty of time for research.

Accordingly Weiss arranged that I should be appointed Instructor in Zoology at a stipend of $3500 and I was to coordinate the teaching program in the Developmental Biology Sequence.

That was the Fall of 1948. How did Chicago differ from Hopkins?
When I went to Chicago, the University was organized into The College and the Graduate School. The faculties were separate. Dr. Weiss' position was that of professor in the Department of Zoology, which, along with Botany, Biochemistry, Medicine, etc., was part of the Division of Biological Sciences. Each Professor in Zoology ran his own show so far as the education of his graduate students was concerned. Dr. Weiss had no seminar program such as Willier did. Once admitted to the Division and to Weiss' lab, students were pretty much left on their own.

Dr. Weiss was in Europe during much of the year that I was at Chicago so I had little interaction with him. I resumed work on feather tracts and published an abstract showing that delayed healing of ectodermal wounds made in the wing bud resulted in failure of organization of the dermis and often the skewing of feather tracts in the direction of the wound. Weiss liked this very much for the movements of cells in wound healing was of great interest to him.

I left Weiss' lab after only one year, for I could not afford to raise my family in Chicago on the salary I received. Weiss offered me an additional stipend from his grant, but it was insufficient.

Then you moved to the Biology Department at Marquette University?
Yes, Marquette was willing to lend me the money for the down payment on a house, pay my moving expenses, and give me a much larger salary than I had in Chicago. This move was the only way I could take care of my family in the long haul.

Could you talk about your views of the contributions of other people who began to work on the AER after your 1948 publication?
Edgar Zwilling picked up on the AER very quickly. In his laboratory at Storrs, CT, he had access to a wingless mutant, which he examined as soon as my 1948 paper came out. In this mutant, the AER formed, but degenerated shortly thereafter. This observation confirmed my finding that the AER is a structure of some developmental significance. Zwilling then went on to show that the defect was in the mesoderm.

You know the problem of the mechanism of wingless is still not solved; we still don't know what the defect is.
Yes, that is true. During the 50's Zwilling went on to do several other significant experiments. He picked up on papers by Moscona showing that you could separate embryonic mesoderm from ectoderm and then recombine them. The next thing Zwilling did, which was important but incomplete, was to reverse the apical ridge on the mesodermal core 180 degrees. He said that the limb developed normally.

The skeleton, I think.
Yes. He was correct, of course, in saying that the anterior-posterior polarity formed normally. But what he failed to do was to allow his specimens to develop long enough to analyze the dorsoventral polarity of the limb, which, as we later showed, was reversed with respect to the polarity of the mesoderm.

Rodolfo Amprino, a distinguished Italian anatomist, proposed that the AER arises simply from the accumulation of ectodermal cells at the apex of the limb bud as a consequence of the distalward sliding of the dorsal and ventral ectodermal faces of the bud. He presumed that cells of the ridge slough off and have no significance for outgrowth of the limb. We showed this to be untrue, for marked cells (quail) grafted proximal to the ridge do not enter the ridge, and marked ridge cells persist during outgrowth of the limb. Soon, too, experiments in Zwilling's lab and mine showed unequivocally that the AER acts as a non-specific inductor of the outgrowth of competent limb mesoderm.

I gather that other people who were attracted to work on the AER knew each other and communicated about it?
Yes, especially people from my lab and in the laboratories of Zwilling, Amprino, and Eugene Bell.

That was a very controversial time.
Notably, Bell created quite a stir when he reported that young limb bud mesoderms grafted to the body cavity or to the flank of a young host embryo sometimes formed limbs showing all skeletal parts in proximodistal succession. To check this discrepancy, Mary Gasseling

Fig. 3. Physiology Laboratory of the Marquette University in the early 1950s. *John Saunders is in the middle with the pipe.*

and I went together to Bell's and Zwilling's labs and repeated the experiments. It turned out that such grafts, carefully prepared and examined by all of us, formed successive limb parts only if the grafts were made to young hosts and became covered by flank ectoderm competent to form a ridge.

What other early investigators can you think of? Was Madeleine Kieny on the scene? Ursula Abbott?

Madeleine Kieny did things which were really very important but that did not initially receive much notice. She showed that very young prospective limb bud mesoderm, minced or otherwise morphologically distorted, and grafted to the flank of a young chick or quail host, would induce an AER in host ectoderm that healed over the graft. The graft then formed a limb, axially organized with respect to the polarity of the host, entraining host flank mesoderm into the resulting structure. She then did several experiments this way, determining thereby the temporal and spatial limits of the ability of the ectoderm to form an AER and of the mesoderm to form a limb. Cecelia Reuss and I, unaware of Kieny's results, carried out somewhat similar, although less dramatic experiments that confirmed her results. I was pleased to acknowledge Kieny's pioneering work at the Seventh International Symposium on Limb Development and Regeneration in Aussois, France, last May. Our collective results were much extended and refined in Jeff McCabe's laboratory.

Meanwhile, in Ursula Abbott's lab, Paul Goetinck studied the eudiplopodia mutant in chick. Such mutants typically show an ectopic AER that arises proximally on the limb after the primary one and induces an ectopic outgrowth of the terminal parts of the limb.

However, you were studying feather development all the time while you were doing other things.

Yes. I wrote the shoulder tract paper at Marquette. And, yes, Jack Cairns and Mary Gasseling and I studied feather development extensively.

Jack Cairns is a man whose name is essentially forgotten.

I try to bring up his name every time I can. Jack was a most meticulous observer. He could tell you where a feather came from by looking at it. I think we benefited enormously from Jack's keen powers of observation. For example, we grafted prospective thigh mesoderm from a 3 and 1/2 day leg bud to an excavation of similar size in the wing bud of a similar host. The grafted mesoderm was covered by healing ectoderm and, in favorable cases, the host hatched and grew to adulthood. In every case, the graft induced morphologically typical thigh feathers in the wing ectoderm. Jack examined our specimens meticulously and was able, in one case, to find a chimeric feather at the border of the graft site. One side of the feather vane showed a distribution of pennaceous and fluffy structures characteristic of a particular thigh feather follicle, and the other side showed a barb distribution of a particular shoulder follicle. Clearly there exists a remarkable specificity of signaling between mesoderm and ectoderm within the feather follicle itself. Events leading to this remarkable specificity must begin long before the feathers emerge.

Why don't you talk a little bit about the ZPA?

We recognized in the mesoderm of the 4-day chick wing bud, near its junction with the body wall, a zone of massive cell death (apoptosis). At early stages, this zone is located just proximal to the beginning of the AER. We asked whether a graft of cells from the future apoptotic zone beneath the thickened AER would permit the persistence of the overlying anterior apical ectodermal thickening.

I should tell you how I remember how it happened.

Yes. [Laughter].

Mary Gasseling and I were putting cells from the Posterior Necrotic Zone (PNZ) into culture. Then she would graft them to various places on the limb and one of the places was under the apical ridge. In the morning, we checked to see if the cells had died or not. (Whether or not they died was seen to depend on the stage of the donor embryo prior to grafting). We noticed one of the limb buds with a graft under the apical ridge was broadened apically at about 24 hours after the operation. We had you look at the limb and you said let's do this again. I continued with the PNZ culture work and Mary started grafting posterior limb bud pieces under the ridge. It was very exciting. Serendipity as you have said.

Why did you call it the zone of polarizing activity?

Well, the reason was first of all that, regardless of where the graft was placed along the anteroposterior length of the AER, the broadened tip continued to grow and give rise to duplicated terminal limb parts, particularly the hand parts and distal part of the forearm. The anteroposterior polarity of the ectopic parts depended on where the graft was placed along the rim of the bud. When we placed the graft under the AER apically, the limb formed a bulge rostral to the graft, a supernumerary limb of right-hand asymmetry occurred. If the graft were placed rostrally under the ridge, an ectopic bulge arose posterior to the implant, and from this supernumerary limb parts of left asymmetry formed.

So the reason for the name then is …

The fact that the anteroposterior polarity of the duplicated limb parts is determined by the position of the graft. In each case, the posterior side of the induced supernumerary limb parts faced the graft site. We called the mesodermal site from which the graft was taken the *"Zone of Polarizing Activity"* simply as a convenient designation, for it seemed to play a role in determining the polarity of the anteroposterior axis of limb parts.

At the same time the ZPA experiments were going on in your lab, you and Mary were exploring the effect of rotating the distal limb bud tip on limb development. Why were you doing these experiments?

We asked the question *"when does the axial organization take place with respect to the anterior-posterior axis",* which is clearly differentiated in the chick wing. So, we severed the tip of the limb bud and rotated it on its stump 180 degrees with respect to the A-P axis. Would the limb tips formed from the rotated tip retain their original polarity or would they regulate and form normal limb parts? We found when we did this that the limb would develop according to its original axis of polarity in some cases. But in most cases, we found that not only did we get a reversed limb tip, but also found a duplicated right hand postaxially. We really couldn't quite understand how this was happening. There must have been some effect of the postaxial stump on the rotated preaxial tip. About this time, Zwilling came out with his notion of an apical ectoderm maintenance factor, which came from his analysis of the duplicate mutant in Landauer's laboratory. Embryos of this mutant showed broadening of the wing or leg tip apically. Zwilling did grafting experiments to show that the duplicate condition is determined by the mesoderm. So he said there must be something about duplicate limb bud mesoderm that maintains the preaxial apical ridge in a thick and inductive configuration and thus leads to the production of supernumerary parts. We used similar reasoning to interpret our experiments; we said that when the limb tip is rotated, something must move from the posterior mesoderm of the stump into the rotated tip, and it must be this maintenance factor that causes the rotated preaxial to form the duplication.

One of the things that struck me was that the tip rotations was actually the same experiment as the ZPA grafts. It was interesting that you did the ZPA and tip rotation experiments almost side by side and interpreted them differently. Recent work from Zeller's lab has proposed that apical ridge maintenance is an effect of Sonic hedgehog's action on the adjacent mesoderm. That fits with your original thinking in a nice way. What was your reaction when Tabin's lab reported their work showing Sonic hedgehog to be the molecular basis of the ZPA?

I was thrilled. At last we were beginning to see something that made sense. At least we were beginning to arrive at the nature of the signal from the ZPA. We had talked all along about the *"conversation"* between the ectoderm and mesoderm; the conversation between this part and that part. We were really naming signals of which we had no inkling when the original experiments were reported. Now we are beginning to understand the molecular signals that operate between ectoderm and mesoderm. Then when FGFs came into prominence in limb research, I was even more thrilled. Now we are on our way. To me it was a great source of satisfaction. Made me feel that what I did wasn't something to just be forgotten, and it has turned out to be very useful.

An area of research that you brought to people's attention was cell death during normal development. How did that start?

It started back in Willier's laboratory where there was great concern about the origin of pigmentary patterns. It was clearly recognized, from the experiments of Mary Rawles and Hans Ris in Willier's lab, that the source of pigment cells in the chick, is the neural crest. Both before and after my military service I used the dye Nile Blue to enhance the visual field in my chick operations, no matter what the experiment I was doing, whether it was on the ridge or on feather tracts. There seemed to be a pattern of cells staining with the vital dye other than those in the posterior necrotic zone that we have already discussed. I thought some of them might be pigment cells, for I saw them *in ovo,* notably above the neural tube. Some of these cells at later stages seemed to have vacuoles containing melanin granules when examined supravitally. I wondered if I was seeing stages in the differentiation of melanocytes. I gave a paper on this in Ithaca, showed the pictures, and just raised the possibility. Zwilling, calling from the back of the hall, said that many of the cells I showed looked a lot like the ones that Honor Fell described in the *"opaque patch"* of the 5-day leg bud.

Fig. 4. John Saunders at Marquette University in the 1960s.

Fig. 5. The Seventh International Symposium on Limb Development and Regeneration, Aussois, France (May, 2000). *The people in the photo all participated in the First International Symposium on Limb Development and Regeneration-Grenoble, France, in 1992. From left to right: Richard Hinchliffe, Ursula Abbott, Jacqueline Géraudie, John Saunders, Danielle Dhouailly and John Fallon.*

This was a paper that Honor Fell reported cell death in the forming knee joint?
Yes. I began to be pretty embarrassed by my hasty thoughts. With this recognition, I began to look at the opaque patch and that made me recognize that in the chick embryo there are also zones of cellular degeneration in the posterior edge of the wing bud and on the anterior edge of the wing bud of the chick embryo. That led Mary Gasseling, Lilyan Saunders and me to map the distribution of the degenerating cells and to do experiments to learn when cell death is programmed during development. This latter, of course, was later explored by William Held in my laboratory and by you and your students at Madison.

These cells are occurring in the mesoderm along with pre and postaxial edges of the bud in the chicken.
Yes, but not in the mouse or the quail, and not in other vertebrate species so far as I know.

So that's an important point, that the same patterns of cell death are not universal among the amniote limbs and are different in different amniotes. What do you think the utility of the anterior and posterior cell deaths would be?
Possibly the patterns of cell death cooperate with patterns of cell proliferation in the development of the contours of the limb and in joint formation. Death clearly plays a significant role in the deletion of the human tail bud, as you have shown, and in patterns of interdigital death in the footpad of amniotes. You began to study apoptosis in my lab, and have since continued, along with many others. However, I really look forward to further progress in the matter of molecular events involving the morphogenetic role of cell death in the limb.

To change to a different topic. How have the various pressures for funding, publishing and so forth changed in the 60 plus years that you've observed science? What effect do you think the changes have had on science?
They have had various effects. One effect is that the demands of brevity has curtailed the ability of authors to publish in grammatically and rhetorically correct English. In an earlier era the insertion of the phrase *"data not shown"* would probably have led to prompt editorial rejection of a manuscript. Today controls are not properly shown; I think that the intelligibility of papers is less than it used to be. The pressure for funding means that one must publish too rapidly; short papers come out in a hurry without citing all the evidence. I wish this was not the case.

I want to return to Paul Weiss. My graduate students have been reading his *"Principles of Development"* in the lab and it has a currency today that is amazing considering it was published in 1939.Waddington's books also can be read with currency. What do you think the difference is of those investigators? Why are their ideas so alive to us today?
Their ideas are still legitimate, still to be pursued because they deal with significant syntheses of data that emerged in the construction of concepts that are global. Science properly done is the ordering of information into general themes, general ideas, general concepts and the recognition of the interrelatedness of facts. The recognition of major concepts gives you insights that are important into the development of new experiments, thus leading to new insights. An area where this same kind of thing seems to be happening is evolution and development. But at other levels of organization, such as organogenesis, I don't see the emergence of significant concepts. I don't see any unifying

concepts to apply to say limb development. We have the old concepts that apply but do we have any unifying ideas that tell us where to go next other than just pick up mutants and check them out. That's why I can't answer about where I think organ development research, such as the limb, is going. As it is going now, we are just gathering mutants and trying to make schemes, trying to make flow charts. I have diagrams on my computer on gene actions but I can't remember them unless they are in front of me.

Most of us have definite ideas about your contributions to developmental biology. However, I would like to know what your perception is of your contributions.
I have made contributions to certain areas that we have already recognized, namely the significance of the apical ectodermal ridge. Secondly, the role of the ZPA. Thirdly, the ectodermal control of dorsal ventral polarity in the limb. Next, the organization of feather patterns. And finally, the recognition that cell death plays a significant role in development (building on the work of Glücksmann, Fell and others). I think these have all provided a basis for analysis of the role of patterns of gene activity in the elaboration of morphogenetic patterns. The apical ridge, as a signaling mechanism, has turned out to be very productive. I guess it formed a basis for the exploration of the role of changing patterns of gene activity and the realization of morphogenetic patterns. I think this is true of all of the things we have discussed; it is true of the AER; it is true of the ZPA; certainly it is true of the recognition of control of dorsal ventrality. These have all been explored by molecular biologists with the result that we have elaboration of considerable data relative to morphogenesis. Cell death has become a big thing but, in my view, has not been exploited as it could be in development. Thus, for example, the cellular and intracellular events that have been elucidated in cell death in the fly and in lymphoid cells have not been applied to analysis of many kinds of cells that Glücksmann initially categorized as involved in what we termed *"morphogenetic cell death".*

What was the most fun in the lab?
I think the most fun, really, was discovery of the ZPA and especially what preceded it - the rotations of the apex. Those were great fun experiments.

Was the fun in the surprise?
Yes. It was fun to see something no one had predicted. What we saw led us to make predictions as to what would happen when you appose postaxial tissue to preaxial tissue in any of dozens of combinations, most of which we never published, but all of which now fit into the concept of a morphogenetic effect of the ZPA.

I have to say I've really enjoyed this time with you.
This session has been a very rewarding one for me. I am very pleased that the things that we pioneered retain their ability to stimulate new experiments in the molecular era of developmental biology.

KEY WORDS: *apical ectodermal ridge, zone of polarizing activity, serendipity.*

Summary

John W. Saunders Jr. is an outstanding contributor to the field of Developmental Biology. His analyses of the apical ectodermal ridge, discovery and study of the zone of polarizing activity, insights into cell death in development, and analytical studies of feather patterns are part of a legacy to developmental biology. The body of his published work remains central to the understanding of limb development and is a major reason for the premiere place that the developmental biology of limbs holds in our research and teaching today.

Beyond these things known to nearly everyone, there is John's role as teacher that is equally impressive. His one-on-one style, in small groups or from the podium is engaging, encompassing, and above all else, enthusiastic about the study of the development of living things. His love of developmental biology comes through to students of all ages and is inspirational. And, of course, inimitable charm accompanies the substance of any interaction with John. He still teaches in the Embryology Course at MBL Woods Hole. Recent students say that hearing his lectures and his involvement in the laboratory are highlights of the course. His continued knowledge of science and delight in new advances is a model for students to follow and they recognize it.

John Saunders is a scientist and educator *par excellence*. His contributions have stood the test of time. His personal interactions with colleagues and students have enriched their lives in innumerable ways, large and small. His is a lifetime of outstanding achievements. In this interview, he reflects on his six – going on seven - decades in science and his personal enjoyment of recent advances in Developmental Biology.