Exploring the epididymis: a personal perspective on careers in science

Terry T Turner

Asian Journal of Andrology (2015) 17, 704–707; doi: 10.4103/1008-682X.145432; published online: 26 December 2014

Science is a profession of inquiry. We ask ourselves what is it we see and why our observations happen the way they do. Answering those two question puts us in the company of those early explorers, who from Europe found the New World, and from Asia reached west to encounter Europe. Vasco Núñez de Balboa of Spain was such an explorer. He was the first European to see or “discover” the Pacific Ocean. One can imagine his amazement, his excitement when he first saw from a mountain top that vast ocean previously unknown to his culture. A career in science sends each of us seeking our own “Balboa Moments,” those observations or results that surprise or even amaze us, those discoveries that open our eyes to new views of nature and medicine. Scientists aim to do what those early explorers did: discover what has previously been unknown, see what has previously been unseen, and reveal what has previously been hidden. Science requires the scientist to discover the facts from among many fictions and to separate the important facts from the trivial so that knowledge can be properly developed. It is only with knowledge that old dogmas can be challenged and corrected. Careers in science produce specific sets of knowledge. When pooled with other knowledge sets they eventually contribute to wisdom and it is wisdom, we hope, that will improve the human condition.

To those gathered for the Sixth International Conference on the Epididymis, it goes without saying that the epididymis is an intriguing biological puzzle. Mysteries remain concerning the organ’s vasculature, the peritubular and interstitial cell populations, epithelial cell biology and function, the tubule’s intra-luminal microenvironment, and the spermatozoa contained in that environment, not to speak of the interactions between all of them. The organ reminds one of a quote from Winston Churchill, the Prime Minister of the United Kingdom during World War II, regarding the foreign policy of the Soviet Union at that time: it was a “puzzle inside a riddle wrapped in an enigma.” That certainly describes the epididymis.

The epididymis (from the Greek: on, at, or attached to [epi-] the twins [didymis], “the twins” referring to the testes) has been mentioned at least since the time of Aristotle; but nearly a thousand years later, in 1668 when De Graaf made his famous drawing of the dissected male tract (Figure 1),¹ the function of the epididymis he had wonderfully dissected down to a single tubule was still unknown. De Graaf’s work predated Leeuwenhoek’s discovery of spermatozoa, so he had no concept of the organ’s true biological function. All he and other scientists of the time knew was that the testes produced a vital substance required for production of a pregnancy in a female. De Graaf speculated that the long, single tubule of the epididymis was necessary for the “completion” of that substance. Now, approximately 250 years later, we know his conjecture has proven true.

The vital substance from the testis was soon proven to be spermatozoa, and research, especially of the last 100 years, has shown the epididymis to be necessary for four classical functions: (1) the maturation of spermatozoa as they transit the epididymis, (2) the concentrating of spermatozoa in the epididymal lumen, (3) the transport of spermatozoa from the caput region to the cauda region, and (4) the storage of spermatozoa in the cauda.² Either directly or indirectly, every presentation at this conference will fall under one of those categories. Presumably, at least one of those functions has attracted the interest of each person at this conference including the trainees, those graduate students, postdocs, medical students, and medical residents who are just beginning their careers. My question to them is, why? Why are you interested in the epididymis? Is there a reason beyond that you happened to stumble into a laboratory that was already focused on the organ? There should be a reason. There should be something about this puzzling organ that genuinely interests you, though that reason that will not necessarily be the same for all of us.

For myself, I became interested in reproductive biology through a concern about world population growth. In the 1960s and 1970s, when I was in college and graduate school, concerns had risen about the rapid expansion of the world population over the last 100 years (Figure 2), an expansion that was projected to continue into the future. In short, given the rapid advances in medicine over the last 100 years, the world population had become too fertile for its own good. It is certainly true that infertility is a serious issue to afflicted couples wishing to have a child. Research into the basic biology of infertility is also important; but in the overall population the impressive reduction in death rates, especially childhood death rates, during the 20th century has resulted in a population growth that is unsustainable in the long run. Contraception, the voluntary regulation of fertility, has seemed a rational approach to the population problem, and I was attracted to reproductive biology as part of the mechanism for achieving that goal. I became interested in the male system, the testis and epididymis, specifically, because little research had been
done there relative to the female system. That is my answer when I am asked why I became interested in the epididymis, and that interest in contraceptive development has remained the underlying footpad upon which my 40 years of research in the epididymis was built. To the trainees here I say I hope you have underlying reasons for being interested in the research you do. We all need a foundation that will sustain us during the difficult days when our research fails to progress as we had hoped, or our results do not clarify the issue as we had expected.

My second question to the trainees present is, once your interest is focused on a topic like an epididymis, what, in a broad sense, is it that you expect a scientist should be doing? Is it to fill in little pieces of data to fit a preexisting idea? Is it merely to confirm, to conform, to fit into a largely preexisting picture? There is some of that in all of science, and certainly, establishing the details of a process and confirming results are important, but from my perspective the driving aim of every scientist should be to discover something previously unknown, to see something previously unseen, or to reveal something previously hidden. When we do that we are living a scientific life to the fullest, we are taking chances and putting ourselves in the ancient company of adventurers and explorers who left their homes and known places to see what was on the other side of the far horizon, to see things that had never been seen before by anyone they could imagine and to discover things that had previously been unknown.

One of those explorers was the Spaniard, Vasco Núñez de Balboa. He was the first European to see or “discover” the Pacific Ocean. In 1513, most of his European contemporaries having arrived in the Americas thought they had arrived in India or maybe even China, at least somewhere in Asia; but in September of that year Balboa reached the top of a mountain in the chain running down the length of the Isthmus of Panama. When he looked over the top of the mountain peak what he saw on the other side was not a large continent to be explored, but a vast ocean, the Pacific Ocean. It was an unexpected thing, and it is easy to imagine his eyes widening, his jaw dropping, and his thinking, “Oh, my! Look at that!” No one from his world had ever seen what he was seeing. Outside of rumor and speculation, no one had expected it, yet here he saw something that would lead to more exploration and more discovery. It was what can be referred to as the first Balboa Moment, an unexpected finding that excites the explorer or scientist and leads to further investigation and revelation.

Scientists with experience have surely had their own Balboa Moments, and I encourage each trainee here to be alert for his or her own. They will sustain and encourage you over the years to come. For illustration of the kinds of things to watch for, I will give three examples from among several that were important to me.

My first Balboa Moment happened when I was still in graduate school. I was learning to use a scanning electron microscope (SEM) when one day I mounted a small, prepared sample of caput epididymidal tissue. I had little hope of seeing anything interesting because I knew SEM is used to look at the surface of things, not their interiors. One typically cannot see into pits, tunnels, holes or tubules because of a limitation in the SEM technique. Imagine my surprise when I turned on the scope, ran up the magnification, and looked into the scope’s eyepieces. There before me was the view of a grassy canyon.

Figure 1: The 1668 drawing of Regnier De Graaf illustrating his dissection of the human testis and epididymis. In the enlargement panel note that De Graaf had dissected the epididymis down to a single tubule. Not knowing of spermatozoa, he still believed the epididymal tubule helped in some way to complete the substance from the testis that was vital for the induction of pregnancy in the female. Reproduced from De Graaf R\textsuperscript{1}.

Figure 2: A projection of world population growth since the beginning of the human species. Relatively stable for many thousands of years, the population began to expand rapidly near the beginning of the twentieth century (1900 arrow) and has multiplied roughly seven fold since then (2014 arrow). In the absence of major efforts at contraception or catastrophic human disaster this expansion is projected to continue into the future.
stretching away into the distance, its walls steep and with boulders strewn in the grass. I quickly realized I had happened upon a view of the interior of an epididymal tubule. Somehow, the sample’s orientation during preparation and its orientation in the scope were such that I was getting a “sperm’s eye” view of the tubule lumen, the microvilli of epididymal epithelial cells being the “grass” and the “boulders” being what I suspect we today would call epididymosomes. I could alter the focus and orientation of the sample to achieve an impression of zooming along the tubule interior just above the level of the microvilli (Figure 3), and I thought, “Wow, look at that!” The year was 1970 and I was convinced my eyes were the first to see the terrain of the epididymal lumen. The idea excited me. It was my first Balboa Moment.

That small episode makes the point that a Balboa Moment is primarily an experience that excites and motivates the investigator. Whether it excites others or changes fundamental understandings is of secondary importance. Scientists had known of epididymal microvilli since the late 1800s, so my “sperm’s-eye view” of them revealed nothing other than a unique perspective; still, the thrill of having had that view spurred my interest in the biology of the epididymis and encouraged my research. Other Balboa Moments can be of a similar scale, something important only to the investigator though, clearly, at their best they can be important findings that change how others view the organ or the processes within it.

Another Balboa Moment occurred to me almost a decade later, and this one, while only a simple experimental result, was somewhat more important. In 1979, investigators had long known that caput epididymal spermatozoa had no capacity for progressive motility, that they developed that capacity during their transit through the epididymis, and were fully capable of progressive motility in the cauda. What they also believed was that spermatozoa in the cauda epididymidis were actually swimming in the tubule lumen. That observation was totally unexpected and, importantly, implied something new: the epididymis produces an intra-luminal microenvironment that suppresses the motility of cauda spermatozoa that are fully capable of expressing motility. That simple observation was a Balboa Moment because it surprised me, excited me, and led to several years of research by our laboratory and others focusing on the regulation of intra-luminal sperm motility.1–7

That example of a personal Balboa Moment was based on relatively simple data derived from sperm motility scores. A third example comes from a far more complex data set. In the early 2000s my laboratory had determined the mouse epididymis could typically be dissected into 10 anatomical segments. We ran multiple microarray analyses of gene expression in all 10 segments and compared the segments’ data sets by using principal component analysis (PCA). PCA examines data variability in two or even three dimensions and identifies data sets that are most similar through those that are least similar.8 A two-dimensional PCA heat map (Figure 4) was how my colleagues and I originally viewed the data. For me, it made a Balboa Moment.

Using the colors white, yellow, and red, the heat map showed which segments were very

Figure 3: Scanning electron micrograph of the epididymal lumen giving a “sperm’s eye” view of the lumen wall and the epididymal microvilli that line it. In 1970, getting this unique view of the “grassy fields” lining the tubule lumen spurred my imagination and became my first Balboa Moment.

Figure 4: A two-dimensional heat map illustrating a principal component analysis of the gene expression data from the 10 different segments of the mouse epididymis. Data sets are indicated as being very similar (white), somewhat different (yellow), and very different (red). The analysis shows that gene expression patterns in Segments 1, 2, 3, and 7 are unique unto themselves. Gene expression patterns in Segments 4, 5, and 6 are very similar, as are the patterns in Segments 8, 9, and 10. The illustration implies that the 10 segments of the epididymis form 6 sectors of unique function based on similarities of overall gene expression.
alike (white) to somewhat different (yellow) to very different (red). The PCA analysis of the expression of all 36,000 genes in multiple samples of all 10 epididymal segments of the mouse showed that Segments 1, 2, 3, and 7 had overall gene expression patterns that were unique to themselves. Segments 4, 5, and 6 shared similar gene expression patterns, as did Segments 8, 9, and 10. If one allows the speculation that overall gene expression patterns have functional meaning, then our PCA analysis meant that while the mouse epididymis has three traditional regions (caput, corpus, cauda), and 10 anatomical segments, it has six functional sectors related to sperm maturation and storage. This finding added yet another level of complexity to the epididymis and led to further research into how such segmentation of gene expression might be regulated.

Similar experiments in the rat showed its epididymis to have 19 dissectible segments, and 9 functional sectors. Interestingly, there was somewhat more statistical overlap or “blur” between the more numerous segments in the rat than in the mouse. On that admittedly thin evidence, one might speculate that the more segments a species’ epididymis contains, the less distinct the differences will be between them. We have counted the segments in the rhesus monkey epididymis to be approximately 30 and a healthy human epididymis from a male within his reproductive years to be approximately 50. If the speculation about increased segment numbers leading to less clear segmental differences is correct, then the functional sectors in the human epididymis may be very gradually applied and thus, indistinct. Only future research will show whether this is correct.

I relate those three personal Balboa Moments as examples, not because they should be important to anyone else, but because they were important to me at their time and inspired me to further investigation. I urge trainees and senior investigators alike to seek your own Balboa Moments. Importantly, they must be based on correct results, which means data must always be confirmed by replication. This is especially true when experimental results do not conform to preconceived ideas. Importantly, when confirmed data do not show the expected result then the validity of the expectation must be examined.

A sign that used to hang in my laboratory says, “Challenge Dogma” (Figure 5). It means that one must stand by confirmed results even if they conflict with previously accepted “facts.” The facts we are taught are merely the best available knowledge at the time. Passing years and more advanced research may eventually show that some facts are not facts at all. That is how science progresses, not by hoping for evidence that merely supports preexisting notions, but finding new information that may suggest otherwise. Importantly, the brave scientist uses those data to challenge dogma, and it is he or she who helps most with progress and innovation. Let that progress and innovation be your aim.

Finally, if we follow the scientist’s goal of discovering things previously unknown, seeing things previously unseen, and revealing things previously hidden, we join those explorers of ancient days who made fresh discoveries about the larger world. Our discoveries may be more subtle, yet not necessarily less important. Your role as a scientist studying the epididymis or, for that matter, any other part of nature, is to do your experiments carefully so that your results reveal facts as we can best know them at the time. Those facts become knowledge and knowledge summed from many sources becomes wisdom. As we all hope, it is wisdom that truly improves the human condition.

ACKNOWLEDGMENTS

Many thanks are due to colleagues who over many years have engaged in the helpful discussions and collaborations that have led to this article, but special gratitude goes to colleagues in the reproductive sciences at the University of Virginia, especially Stuart S. Howards, Jeffry J. Lysiak, and Barry T. Hinton.

COMPETING INTERESTS

The author declares no competing interests.

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