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How to write an Invertebrate Anatomy Book

Adrian Horridge

During a long life, most of my students and colleagues researching on invertebrates were Mechanists, interested in how animals actually worked inside; like machines or circuits. This is not the same as Function, which expresses adaptation for an abstract purpose such as ‘stomachs are for digesting’, or ‘brains are for co-ordination and initiation’. Mechanists use equipment to isolate components, record activity, take things apart and analyse interactions. That is often a very attractive activity that may lead into the anatomical foundations.

Three or four of my people, however simply wanted to describe ‘what was there’. Two post-docs were American electron microscopists, and two were English. The Americans arrived ready trained in the early 1960s, and introduced best practice at a time when we had just installed our first electron microscope in our Marine Laboratory, so almost anything one examined from the sea would yield fresh and sometimes startling results. About 1965 one of the English, Ian Meinertzhagen, started to cut serial sections of the axons of the photoreceptors of the blowfly’s eye, using a thin-section cutter and material embedded in resin. Today 2014, as I write, Ian is still cutting serial sections, nowadays of the eye of *Drosophila*, with great success. He and his group are working their way down through the optic lobe and re-assemble the exact anatomical circuitry of the medulla, where the peripheral serious business of vision is conducted. The other Englishman, Mike Bate, started about 1970 to follow the successive cell divisions in the developing egg of *Drosophila* exactly, and tracked them into the first signs of different tissues, including nerve cells. Eventually, after years, the whole cell lineage became an essential reference work for molecular and genetic studies of development in the most studied insect.

I watched it happen. Within a laboratory mainly devoted to electrophysiology of arthropod vision, were two researchers of a different species, different time scale, different ideas about permanent knowledge, who needed not much support except time; time which drew out into years, decades, a lifetime. They were motivated; they were bitten by the bug of exact anatomy, and I can explain exactly how that bug can be cultured. In their case, it grew in a rich medium of colleagues of diverse interests, secure if not substantial salary, relaxed working conditions, ancient stress-free scenic surroundings and libraries of journals and beautiful books. Briefly, the whole effort must be soothing for the soul. All it needs is an initiator at the right time.

In my case, after finals, when I got home from the Canary Islands in early Sept 1949, waiting for me was an offer of a PhD studentship. This would provide enough support for three years’ research on a topic of my own choice. When I turned up at the lab, the chief technician gave me a key to a room – and that's all I got. The room was quite empty.

The Zoology Department in Cambridge was stuffed with Fellows of the Royal Society and was committed to the experimental approach. James Gray, the Professor, was interested in the reflex control of movement. Arthur Ramsay was the world expert on the excretory system of the earthworm. Wigglesworth had a strong group working on insects. Eric Smith was an expert on the

nervous system of annelid worms and echinoderms. Carl Pantin was the world's expert on the nervous system of the sea anemone, such as it was. Pringle and Pumphrey had already developed the art of recording from nerve cells in a variety of animals. It was up to me to make the next move if I wanted to choose my own topic. This was a challenge; none of these academics were there until term started.

As a research student in Cambridge in those days, if you showed the inclination to make your own way, you stood up on your own feet, then you walked alone or preferably ran. It was a complete contrast from the process of stuffing undergraduates with facts, but I was well prepared by the closure of school in wartime. You found your own topic, searched the literature, and looked for somebody who would nominally supervise you. You could say that the system was designed to pick out those who would do research whether encouraged or not. Self reliance was the key, and one had to pay one's own travel and living expenses out of a small grant. You had to collect apparatus, which you either built yourself or scrounged from somebody who had just finished, or some other member of staff who had stuff in his cupboard. So, having assembled everything you thought you needed, you might start experiments.

About half of the PhD students who could not stomach this tough regime attached themselves to a supervisor, and were given a part of the program for three years on a defined path with a mentor. That would not suit me at all. I had no topic and hoped to find a novel winner, so I went down to the Plymouth laboratory of the Marine Biological Association of Great Britain, which is an independent body funded by the government. This laboratory had two ships with which it explored the western fringes of Europe, the Atlantic Shelf and the English Channel, kept an eye on fisheries and ocean productivity, and supplied specimens to the Universities. The lab was filled with enthusiastic and experienced researchers on interesting animals, and they were willing to talk shop all day. I had discovered this while on a two-week course in Marine Biology. Now a Cambridge graduate, I simply walked in and said I hoped to stay a while. Freddie Russell, the Director allowed me to work there, supposedly on the Cambridge University table, and showed me his cultures of Hydromedusae in his cool sink.

In the specimen store was an interesting item. In the 1920s, Berrill, a Canadian visiting zoologist, had cut off the heads of marine tube worms, *Sabella*, and studied the regeneration until they eventually grew back to normal. He left behind a bottle full of partially regenerated worms. Actually, that was his only result. I hoped to look at the process again and follow the regeneration of the nervous system using electrophysiological techniques and staining the nerve cells.

Working in the Plymouth laboratory was a distinguished Polish medical professor, J. S. Alexandrowicz, who had been Director of the Medical Institute at the University of Lwow, fought with the Polish army during the war, escaped via Russia and Iran, and then landed in England, but was unable to return home. In 1932 he had made the classical description of the innervation of the crab heart. Carl Pantin had found a place for him as a Trinity College gardener, then as an extra staff member in Plymouth, where he produced a remarkable series of papers on peripheral stretch receptors and cardiac innervation in various invertebrates, mainly crustaceans. I owe him a great

debt. When I arrived, he was staining the stretch receptors of *Homarus* and *Squilla* with methylene blue. That is very much an art, with lots of tricks in the technique.

He taught me very well how to handle methylene blue staining of neurons in invertebrate animals, with crabs, *Squilla*, polychaetes and starfish. The methylene blue is first reduced with sodium hydrosulphite until the colour just disappears. Then it can be injected into the animal, which is killed, opened and dissected under a low power microscope. As an alternative, a thin piece of fresh tissue was stretched between cactus spines on a small flat slab of wax, and floated upside down on methylene blue solution. Non-ferrous pins were said to be essential. Sometimes, after about half an hour, beautiful preparations with numerous nerve cells, axons and their connections were revealed. Often there was nothing but blue fog, and Alex would blame the sample of dye as not being suitable for that part of that animal at that temperature on that day. If anything appeared, it had to be drawn with great care under a camera lucida. Specimens could be fixed in saturated ammonium molybdate solution, but it was difficult to clear and preserve them. I usually left the lab at bed time, but Alex would stay on, late into the night, surrounded by dozens of preparations that he examined from time to time, hoping to see something new.

Alex also showed me the literature in the extensive library. The first essential for picking a research problem is to know the literature so well that the gaps are obvious. We found Allen's work on the neurons of the lobster embryo, done in Plymouth in 1894; Alex opened the great volumes of Retzius (1890-6), with the drawings of the crayfish central nervous system; Bethe on crab brain; the isolated effort by Emil Bozler on a medusa *Pelagia*. Some of the best literature was in German, which I read well enough; Sanchez on the leech, *Hirudo*, in Spanish, which I had learned in school. Alex was very proud to bring out works by Orlov, and other members of the Polish school, Zaćwilichowski (husband and wife) , Rogasina, and Ogijewicz on stomatogastric ganglia, insect cuticular sensillae, and wing sensory cells. He made me write down the names of authors and books that I would find in Cambridge. This mentoring can be recognized when you scan through the illustrations in "Bullock and Horridge".

Unfortunately, not a single neuron in *Sabella* would stain with methylene blue.

Back in Cambridge, I went on my motorbike to explore the lagoons on the Norfolk coast, with large jars in my pannier bags. By chance I collected some jellyfish, *Aurelia*. In the zoology lab, (Lord) Victor Rothschild had just purchased some fancy new microscopes with his own money, so I was able to use his phase-contrast microscope to look at transparent jellyfish tissue for the first time. The large nerve fibres of jellyfish are completely transparent but they stood out clearly in the living state under phase contrast. A very lucky break, I thought, and decided to try to record from them. The neurons grow as the jellyfish grows, but jellyfish are not reliable as a source of experimental animals and large ones are difficult to transport. I quickly checked the literature, discovering that nothing much had been done on jellyfish nerves since the great work published by Romanes in 1876, and a paper by Emil Bozler, working at Naples before he had to flee from the Nazis. It was a wonderful opportunity to investigate large living neurons in a phylum that was scarcely known at all.

To record from them, I had to become an electrophysiologist, which in those days was quite new as a topic and all equipment had to be designed and built from scratch. Experiments had to be simple and cheap. I first built a power pack, then a multivibrator stimulator, then a DC amplifier that gave me enormous trouble because it was totally unstable. I got hold of a radar oscilloscope, which had a blue screen and a fast time base that produced circles on the screen, but I changed the tube and time base, so I had an oscilloscope with a slow time base and a green screen that gave a long fluorescence. The Zoology lab had an excellent workshop and plenty of metal sheet, flats and rod, and there was a staff member, Raef Brown, who was full of encouragement and design ideas. There was an electronics technician who made equipment for the staff, but not for students. There was also a good physiology lab across the road – Hodgkin and his assistants – and Willie Rushton enthusiastically instructed me how to make microelectrodes. John Pringle did not supervise students but he let me examine and copy his equipment. I spent 12 months building electronics, looked at lots of animals, and recorded nerve impulses in snails, leeches and others that had accessible nerves. Once you've made all the equipment and discovered the little details of exactly how to do the experiments, you become more confident. One result was that later we trained dozens of students in the new techniques, and they spread over the biological world.

All the equipment was taken on the train to Largs on the Scottish west coast, and then by ferry to the marine laboratory at Millport. There, since I had no money, I camped behind the laboratory for the summer, supposedly on the Director's lawn but actually on a nettle patch. I recorded nerve impulses from jellyfish, correlated them with the beat of the bell, and photographed the synapses in the living state. There was only one nerve impulse for each beat of the bell. Therefore, any other activity of the animal had to be conducted by a separate pathway. Therefore, there was a second nerve net. When stained in the larval stage with methylene blue, it turned out to be so.

I went to Naples every spring to study Hydromedusae and Ctenophores, groups of animals with nervous systems that were hardly known. These animals are planktonic and they were available there because they were brought close to the shore by an upwelling current. Ctenophores are so fragile that, to catch one undamaged, it is essential to immerse a bucket in the sea and waft the animal gently into it, then lift out the bucket to take it into the aquarium to be studied. At that time, there was no other zoologist publishing on their nervous systems.

Consequently, during my 6 years of research in Cambridge I never did any work in the lab in Downing St that led to publishable results, but continued to use the place to build apparatus, read in the libraries, and write, while all my research was done with living specimens in Naples, Millport or Plymouth, with an expedition to the corals reefs of the Red Sea. The Stazione Zoologica di Napoli was supported by universities all over Europe who paid a fee for table rights. (That is, they could send people to work there with their own apparatus.) At that time, the place was one of the great laboratories of the world. From the start, I realised how ignorant I was. At lunch – served in a large dining hall in the lab – you could hear Swedish, Hungarian, Hebrew and other more familiar languages around the table. I got to know many European scientists, and spent

a lot of time working in the magnificent library dating from the 1880s, where there was every possible journal and book and old monograph on marine animals.

To get to Naples, I would catch the Paris train from Waterloo. From the Gare de Nord in Paris, I would go straight to the Opera Comique and standing in the 'gods' at the very top tier I would watch whatever show was on that evening. Then I would rush to the Gare de Lyons and catch the night train to Milan or Rome, then the 'rapido' to Naples. I also took the opportunity to stop off in Lyons, Florence, Pisa, and Rome for a few nights, as a tourist. The Italian currency was so weak that the costs were negligible.

Unavoidably, in Naples one lived and ate with the people. I usually stayed for next to nothing in a huge damp room in the Palazzo Russo on the sea cliffs a little to the north of the Stazione. The flat belonged to a tiny, bent woman of great age and obviously aristocratic descent, who treated me as unspeakable. From this cliff-top room, the view of the castle, fishing and ferry boats in Naples Bay was magnificent, but the room was less inspiring. The mains electricity supply consisted of two bare wires that ran around the walls about 2 m from the floor, supported on porcelain. The room was damp because when the water was turned on, the soft lead water pipe that also ran around the inside of the wall sprayed thin jets across the room from tiny cracks that had developed as it stretched by creep under pressure. Naturally the water was usually off, but it had to be turned on at intervals to see whether any water was there, because there was pressure only at certain hours. The room was also rented to a sculptor, who never appeared, but scattered about were his half-formed monsters made of wire netting covered with plaster of Paris, that shed crumbs on the beautiful mosaic floor. A slight smell of fungus was traced to the mattress, which had mushrooms growing out on the underside.

Unavoidably too, the Stazione Zoologica was an education. The first time I went it was Easter, so the lab was thronged with big names and their assistants. Lunch was cooked and served in the lab. One day the red pepper soup was so hot that my nose began to bleed into the soup. Unable to think of anything better, I let it bleed, then ate the soup but everyone was so busy talking and eating no-one noticed. Opposite me one day a short plump gentleman of about 60 appeared, accompanied by three attractive women. Seeing me, he rose to his feet and solemnly bowed. "Buddenbrock" he carefully pronounced. So I stood and similarly uttered "Horridge". Then, "Are these ladies your assistants" I asked, eyeing the them hopefully. "No", was the answer. "Zeez is my vife. Zeez is my tochter. Zeez is my zecreterry" Knowing they were safe, all the girls smiled at me. Later, he showed me his experiments on the directed swimming and steering by the giant clam, *Pecten*.

There were many nationalities; Swedes studying sea urchin fertilization, Israelies studying squid giant axons, Americans having a tax break, English enjoying the weak currency and the excellent cuisine. Runnström was there with a group of Swedes. Balzer brought a group from Switzerland, and there were several from France and Germany. John Z. Young was there watching the learning process of the *Octopus*, when he was not in Ischia, where he lived with his mistress in a castle belonging to the Stazione, on the cliff at the edge of the beautiful circular volcanic crater.

Later, when I stayed there with Audrey, we could look down from the tower at the loving couple in the pool below. Uncle Buchs also lived in the castle for free. He was an ancient German banker, the elder brother of Reinhart Dohrn, the Director of the laboratory. We also went to Capri, to the house of Axel Munthe, and to the ruined palace of Nero on the cliff top. We also used to go on outings in the town. Eric Smith, who was staining polychaete nerves with methylene blue, and Harold Monro Fox, who did nothing, but mysteriously spoke fluent Neapolitan dialect, together would take me out to the shows at a dirty music hall in a poor district. This was a rough comic show, with bawdy songs for the lower class, incomprehensible but memorable. More importantly, every day I was systematically working through the great volumes of the Pubblicazione della Stazione Zoologica, old zoological works in German by Eimer, Chun, Heider, Haeckel, Bethe, Hanström, Plate, Orlov, Zavarzin, Retzius and the Hertwig brothers, that were on open shelves in the library of the Stazione. The wizened old librarian, I forget his name, became so used to my constant occupation each year that he would lock me in when he went home.

About 1955, Ted Bullock appeared in Cambridge, hoping that Pantin would read and correct a chapter on the Coelenterate nervous system for his great new book that would summarize knowledge of all invertebrate nervous systems. No such luck; Pantin passed him on to me and thereby changed the course of my life. My first response was to rewrite the Coelenterate chapter in my own way, adding all the new information from my five years of research on the topic, much of it in press.

I spent the summer of 1955 at Ghardaqa, Egypt, on the zoologically rich coral reefs of the Red Sea, working on the nervous systems of living coral colonies, sea pansies, and more coelenterates, but that is a whole story in itself. While at the Naples lab, I accepted a tenured job at the Gatty Marine Laboratory at St Andrews University, Scotland, and moved there with my wife. Again, there was a marine laboratory, the Gatty, and a large old library collected by D'Arcy Thompson, to be explored.

Ted wrote a very kind letter, urging me to join him on the book. He had been working on it since the early 1940's, and had finished several chapters on the minor phyla. He wrote those first because they moved slowly and made handy bites. So, when we discussed the matter, he offered me either the arthropods or the molluscs as well as a share in the design, the management of balance and the general editing. I took the Arthropoda, mainly because Alexandrowicz had revealed the possibilities and I was an electrophysiologist.

Right from the start we had a bias towards the neuron as the unit of the nervous system and behaviour. We both realized that the use of microelectodes would spread through the animal kingdom as fast as young men could be trained in the new techniques. Ted had learned a lot from the pioneers in Japan, Kuwabara, Ken Naka, Tomita, Katsuki; and in the USA, Hartline, Kuffler, Grundfest, Fuortes and MacNichol and Wiersma. It was quite obvious that the Americans read no languages except English and that a wealth of anatomical knowledge could be translated and packaged for analysis by the new techniques.

Ted organized the funding from the NSF and invited me and my wife, Audrey, with two children, to California for 15 months. We stayed briefly in Los Angeles, where Ted had set up a laboratory for intracellular recording, managed by Hagiwara and Ladd Tauc. The preparations were the crustacean heart ganglia and squid giant synapse. They opened up the use of microelectrodes on the West Coast. I purchased an 8 cylinder car from Ladd, then we moved to the Berkeley campus for the summer, then rented a house in Menlo Park by the beautiful campus of Stanford University.

Ted and I each had an office at the Centre for Advanced Studies at Palo Alto. We hired 15 artists to do the illustrations for the book and two bibliographic assistants. We could use the typing pool there and the great library on the Berkeley Campus of the University of California, where I could ask for a translator to help with the literature in Polish and Russian. At that time, apart from Japan, almost nothing came from the rest of the world. After the initial collection of content, there was a lot of work refining the book, which had to be a thoughtful work of art as well as a useful guide to the literature. Ted arranged for me to travel in the USA and to Vancouver, to give seminars, and he taught me how to apply for cash. He used to fill out six identical applications and send them to six different agencies. "If you don't ask, you don't get" he would say, with a wink.

Ted found a small publisher who would listen to us on questions of book design. Freeman's publishing office was in San Francisco and Bill Freeman lived in Palo Alto. We went there for dinner. Bill was very keen to publish a high-class book, and Ted found the cash to finish the job. Back in St Andrews in 1961, there was teaching to do, a laboratory to organize, new PhD students, besides the book to finish. I had to catch up with the Russian literature, so it was not long before I was in St Petersburg at the Sechenov Institute, where I barged in simply by telling them that I was hoping to visit and needed a visa (at the height of the cold war). Madame Voskresenskaya was very kind to me; there I first saw a performance of Giselle while discussing neurobiology with her, and that is why her publications were listed in our book.

Ted came over to Europe and we went together to Holland to visit the printers, Enschede en Zonen, one of the best in the world, who print postage stamps and Korans in intricate text for the Arabs. It was printed with cast metal plates on acid-free paper. Bill Freeman had spared no expense to make a fine work of art. Eventually it appeared in 1965, fifty years ago, and currently sells for US$350.

As soon as the book was published, students and professors searched through it for promising new topics and preparations. A whole new industry began, mostly to explore with microelectrodes every large nerve cell; in tube worm, leech, barnacle, crayfish, lobster, locust, *Aplysia*, snail and squid. The Grass Scientific equipment company made a fortune out of it, and funded a Fellowship for Neurobiology at Wood's Hole Marine Biology laboratory. The topics became so popular that there was a backlash from the medics who considered that too much funding was going to useless invertebrates, and in the USA the funding rapidly disappeared. As a result, Ted quickly built up a new team that concentrated on the study of communication by

electric fish. My laboratory at St Andrews started recording from the receptors and neurons of the arthropod compound eye because Vision provided so may avenues for research.

At the Gatty Marine Laboratory after 1960, a succession of students brilliant at research worked happily and productively. In that decade, they were certain to be able to find employment in a University, and morale was very high. I could have stayed until retirement. However, in 1967, while working at Wood's Hole Marine Lab, a cable arrived with an invitation to visit Canberra, Australia. There I was offered a position as one of four founder professors of a new School of Biological Sciences, to be planned, and filled with research of our own choice, with no undergraduate teaching and guaranteed funding.

A number of factors were critical; a growing family, a climate warmer than St Andrews, a career for my well-qualified wife, and the temptation of a different fauna and flora. A critical factor was the darkening economic situation in the UK, with expectation of fewer grants for pure science. Moreover, besides being Director of the Marine Laboratory, I had teaching duties in Zoology, but no academic staff, and had to find most of the funds to support a research team. So, we departed, and took 19 UK citizens, to do it all again in Australia.

Looking back on this account, I see that ten years at Cambridge, getting an education, spotting a living nervous system that no-one had previously recorded from, learning electrophysiology by copying the local experts, and working in marine laboratories where most invertebrates are to be found, suited me fine. It was a fortunate chance that Ted passed his chapter to me when the time was ripe. My early research was essentially selfish blue skies pursuit of jellyfish in the cold northern seas, the corals of the Red Sea, and the rare and fascinating creatures in the upwelling currents of the Bay of Naples. I could afford to work there every year because the locals were so poor. Collaborating with Ted Bullock took our whole family to the New World, and generated a vast surge of opportunity that few experience. The variety of invertebrate nervous systems was laid out to be assembled and published, thanks to a strange coincidence of opportunities, friendships and experiences. I thank them all.