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Careers in Cardiovascular Research

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My career, like those of many others, has changed its course to fit in with other commitments and with opportunities available. Nevertheless, I have ended up still in the same general field in which I started and still doing much what I decided I wanted to do more than forty years ago.

I discovered when I was eight years old that it was possible to spend one's life studying animals. One of my father's wartime colleagues, drafted as was he into 'Research Section 6' of the School of Infantry to study captured weapons, was, in peacetime, a zoologist. I thought this sounded a fine way to spend one's life and from then on I never wavered in my resolve that this was what I should be. When I was 10 or 11, I was given a book called 'The Animal's World' written in the '30s by Doris McKinnon, Professor of Zoology at King's College, London. I still have it and have just looked at it again. The chapter headings such as 'How animals breathe'; 'The fate of a meal'; 'News of the World' constituted an account of animal physiology that I found really gripping. It had no colour plates of course, just some black and white diagrams and a few photographs, but a text written by a scientist who could really communicate to children the fascination of discovering how animals' bodies function. I knew I wanted to find out more.

I was fortunate in the science teaching at school, particularly in the girls' grammar school where I spent the 6th form. There were specialist teachers for all the sciences and most of them were good and enthusiastic. I cleared (just!) the hurdle of compulsory Latin O-level for those trying for Oxford or Cambridge and arrived at the stage of the entrance exams. Oxford's came first; I tried it 'for practice', really wanting to go to Cambridge. There were Biology and Chemistry papers, two (extremely obscure) General papers and a French translation (fortunately, no more Latin). Then came interviews and a practical Biology exam. After this marathon, it was no wonder that I decided that I would accept the place that I was offered at Oxford rather than waiting to try Cambridge. Besides, I had discovered that I could do a course at Oxford which involved three sciences for two years, and one of these in my case could be Physiology, before I went on for a further two years of Zoology. It also gave me the opportunity

for six months 'out' which I spent learning German, since my parents felt no scientist should be without this skill. As things have turned out, I haven't often needed the language scientifically but the visit to a welcoming German family left me with widened horizons and a firm pro-European view.

As an undergraduate, I studied a wide range of subjects, from plant chromosomes to stickleback behaviour, and from the anatomy of the limpet to mammalian neurophysiology. I was still most drawn to physiology, particularly that of invertebrates, about which relatively little seemed to be known. On graduating, I arranged to do a D.Phil. investigating the action of the apparently neurosecretory pericardial organs on the working of crustacean hearts (the extract of these glands had been observed to speed up the hearts and make them beat more strongly). I was to be based in Oxford, but the work was to be done partly at the Marine Laboratory in Plymouth and partly in Naples (I had been awarded the Oxford Naples Scholarship to the Stazione Zoologica there).

It was arranged that I would first spend six months in the Laboratory of Physiology in Oxford, learning the techniques I could then apply to crustacean hearts in Plymouth and Naples. Electrophysiological techniques were then (the late '50s) quite primitive and my own knowledge of them minimal. In my supervisor, Jean Banister's, lab., I action of adrenaline on them. Glass microelectrodes (first used by Ling and Gerard in 1949) were a relatively recent introduction . At first, people pulled

Chunk missing here

them by hand, a difficult art with a 95% failure rate. Mechanical pullers were just coming in and the Lab. workshop agreed to make us one. It was a horizontal one, and somewhat temperamental, but a great advance over hand pulling (which I also tried). Once pulled, the electrodes had to be filled with 3M KCl, there was no glass with filaments down the centre to make this easy. The best way was to boil them in the KCl under reduced pressure, a rather frightening procedure which might ruffle some Health and Safety feathers these days. To record from the beating hearts, I carefully broke off the working end of a microelectrode and sealed it with nail varnish to a coiled length of silver wire. With perseverance and luck, a stable record of action potentials could be obtained from a ventricular cell, even during adrenaline action. This was my first sight of cardiac action potentials (Weidmann's first records had been made only a few years previously). It was quite a thrill to obtain these records and the successful electrodes were carefully labelled and stored for re-use another time. There was one particularly good one ('J') which gave us several good recordings.

After six months of this, my Nauplius stage began in earnest (the Nauplius is the free-swimming larva of the barnacle). I left Oxford for Plymouth, to start on the crustacean project, using hearts of large spider crabs, (Maia squinado). Crustacean hearts are not myogenic, as are vertebrate hearts, but neurogenic, controlled by a rhythmically bursting cardiac ganglion lying within the heart muscle. It soon became evident that I was going to spend most of my time trying to unravel the workings of this neuromuscular system before addressing the question of how the hormone from the pericardial glands acted.

Eight months later, it was time to take up my scholarship in Naples. The Stazione Zoologica, founded in the 19th century by a German zoologist, Anton Dohrn, provided bench space but not apparatus. So on that first journey (by train, of course, 30 hours to Rome and 4 hours on to Naples) I was accompanied by an oscilloscope and the faithful electrode puller, kindly lent me from Oxford. The lab. in Naples (like the city itself) had many primitive features and an atmosphere of exasperating charm. I quickly learnt some essential Italian to get my needs across to the technical staff, all experts in Neopolitan procrastination. 'My' crustacean, a large segmented shrimp called Squilla mantis was in plentiful supply. On my first day I lifted one out of the tank in the corner of the lab. and received a deep gash in my finger. Squilla can close its mantis-like claws with matching spikes and grooves with great force. After that I handled the animals with wooden tongs. It was quite a struggle to assemble even my quite primitive electrophysiogy set-up in Naples, but with help from some visiting American scientists, there to study the Octopus visual system in parallel with J.Z.Young's work on Octopus behaviour, my project went ahead. Squilla, with its segmented body, had a segmented heart where both ganglion and muscle were more accessible than in the compact crab heart. In the end, I was able to stay in Naples for the rest of my D.Phil. work. I ended my three years with enough results for a thesis but without the thesis written. I wrote it during the next year and a half while I was working in London (not an arrangement I recommend involving as it did many evenings and weekends in the university library).

An important career-shaping event had occurred right at the start of my time as a graduate student. In the Oxford Physiology Lab. I met Michael Brown then intercalating a B.Sc. (what now would be an M.Sc.) into his medical studies. While I worked and wrote my thesis, he was completing his clinical training at the Middlesex Hospital. Once he was qualified, we got married. Then came his housejobs- which left me plenty of evenings and weekends to complete the thesis! His subsequent decision to try a further year's research with an M.R.C. training fellowship prompted our return to Oxford. The adult barnacle stage had begun (we have not moved since).

My career then had to become quite flexible. I was fortunate in being also taken on in the University Laboratory of Physiology (where my husband became a Lecturer) first as a Departmental Demonstrator (involving practical class teaching which I have always enjoyed) and after that on a series of grant-supported posts. These I was at first able to hold in various part-time arrangements, the percentage of time worked increasing as our two daughters (born 1966 and 1970) grew older, until I was full-time again.

It was clear to me that I could no longer work on Crustacean hearts. Oxford is too far from the sea for a ready supply of marine Crustacea and the woodlouse and crayfish are too small. Besides, although the Oxford Lab. is concerned with Animal Physiology, one gets the strong impression that only vertebrates (possibly only mammals by some people) are considered to be 'Animals'. My former association with frog hearts had been a happy one and Denis Noble (whose group I now joined)

supported a project on frog muscle. It was evident that voltage clamp data were needed from types of cardiac muscle other than the Purkinje fibre (which because of its favourable geometry, had been the only type of cardiac muscle clamped to date). A sucrose gap method for voltage clamping frog atrial muscle was set up and we found that the preparations showed pacemaker activity when given small depolarizing pulses. Although our preparation was attacked as 'insanitary' in an American review article, we made progess in analysing the underlying membrane currents and moved on to use frog sinus venosus (pacemaker) tissue as well.

My own interest in pacemaking was thus firmly established when Dario DiFrancesco joined our group in the late 1970s. He was determined to voltage clamp mammalian sino-atrial node, which with its small cells and high proportion of connective tissue had proved the most difficult cardiac tissue in which to achieve a uniform voltage clamp. Dario was the first of our group to master the 'small preparation' of sino-atrial node tissue pioneered by the Japanese cardiac scientists, Akinori Noma and (the late) Hiroshi Irisawa. This involved fine dissection under the top power of a stereo microscope of SA node tissue using razorblade tools to cut ultra-fine strips which were then ligatured with the finest available thread (nylon stocking or surgical silk unravelled to its ultimate components) to give a 250μm diameter ball of tissue. This had to be held down by a fine wires and impaled with two microelectrodes. If the cells were still beating and the microelectrodes gave stable, virtually simultaneous records, then voltage clamping could go ahead. (It was by now usually about 6 in the evening after an 8.30 a.m. start). Altogether, it was a highly demanding technique, but all the more exciting when it yielded results and showed clearly for the first time, i_f, the hyperpolarization-activated inward current ('Look! there's that funny current again!') in pacemaker tissue.

Since then, assisted by a fine succession of post docs. and research students, I have maintained my interest in pacemaking, at first continuing with the 'small preparation' and then moving, as have most cellular cardiac electrophysiologists, to patch clamping of single cells. Once again, this has been more difficult for the sino-atrial node than for other cardiac tissues. Those involved with single SA node cells do not, as do their 'ventricular' colleagues, talk of percentage yields, but of whether those (few) cells they isolate beat spontaneously and if so, for how long and, about types and batch numbers of enzymes.

In Denis Noble's group, our experimental investigations of pacemaking have always had the extra dimension and stimulus given by the incorporation of our results into computer models. More recently, as a salutary counterbalance to focusing exclusively on single cells has come his development of network models using parallel computers; the first of these was of the SA node.

I have enjoyed many aspects of my work: the excitement of discovery (when it occurred), the companionship of the lab.- Oxford is particularly fortunate in attracting a stream of talented and interesting scientists from all over the world; the teaching (of which I have done quite a bit); the writing of papers and reviews and now a book, writing is something I have always enjoyed; the meetings, particularly those of the European Cardiac Cellular Electrophysiology Working Group, of which I was Chairman for four years. I was lucky to be able to work part-time when my children were young and to come back into full-time work after that (something which is much harder now in the fierce scenario of competition for grant money). Set against that has been job insecurity and the lack of university and college 'status' for those on soft money.

What have been the best moments of my career? The moment when my D.Phil. examiners asked me to take tea with them- and I felt that they really liked my thesis; the moment when we realised the sucrose gap apparatus was not giving us just noise, but clear records of atrial action potentials; the first records of i_f and other membrane currents by voltage clamp of the 'small preparation'; the satisfaction of isolating healthy, spontaneously beating sino-atrial node cells and being able to patch clamp them and discover more about pacemaking.

One of my lab.'s current projects concerns the investigation of the action of catecholamines on potassium currents in SA node cells. We can show that they cause a faster decay of both components of i_K (i_{Kr} and i_{Ks}) and that this contributes to pacemaker acceleration. Perhaps things do come round full circle: there's certainly a connection there to my much younger self using 'J', the favourite spring-loaded microelectrode to record frog ventricular action potentials during adrenaline action.

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