Lessons on beginning a career in biological research. How the first publications came about.

In Sept. 1949, stuffed with academic knowledge and a PhD studentship in my pocket, I returned to Cambridge to find the Zoology Dept deserted. Busy-busy Mr Drury, chief technician, gave me a key to a room, which was empty but for two stools. My proposed supervisor, Carl Pantin, had gone to Brazil for 18 months (I spoke to him twice in 3 years). I was supposed to find my own topic, build or borrow some equipment, carry out a significant piece of research, write it up and submit it 3 years later. Such was the deep end of the pool at the time.

So, having no better option, I went down to the Plymouth Marine Lab wondering what to do. There by chance I met Dr. Alexandrowicz, a distinguished Polish refugee of about 75, who taught me how to use the vital dye methylene blue to stain living nerve cells in various invertebrates. In 1960, he had published the classical description of the stretch receptors of the lobster, later an important preparation for the physiology of synaptic inhibition. In Cambridge and Naples, Eric Smith was staining the central nervous systems of Polychaetes and Echinoderms similarly, so I absorbed the literature and the technique from these experts. Much of our basic knowledge of invertebrate neuron connections came from methylene blue studies that were pre-war or older, by Retzius, Sanchez, Orlov, Zaćwilichowski, Zawarzin and others, most of it in French, German, Polish, Russian or Spanish, not summarized at the time, and not familiar to English students. I was more of a gadgeteer than an anatomist, but the old literature, when put together with the new techniques that I had learned about, presented endless opportunities to advance our understanding of the nervous co-ordination in lower animals.

1951 *Nature* 167, 732-734.

Earlier, Martin Canny and I had been on holiday on the island of Tresco, in the Scilly Is. On the last day, I collected a plastic bag full of porcelain crabs to take back to the lab in Plymouth, packed in moist seaweed. At the time, I was staining with methylene blue everything that looked promising. Back in Plymouth, Dr Parkes noticed the seaweed, and with a cry of delight picked out a dirty brown tangle of strands which she said was a species new to Britain. She found the illustration of *Asparagopsis* in Harvey's Seaweeds of Australia and insisted that I write a letter to Nature. Such was the first step on my professional path, in the wrong direction, of course.

1952 Birds on Palma and Gomera. *Ibis* 94, 68-84.

In 1949, when I visited Oxford to look for possible places to apply for research, Philip Guiton and I planned parallel expeditions to survey birds in the Canary Is. David Snow went to San Tomé in the same year from Oxford. David Bannerman lent us his 410 gun, disguised as a walking stick, which we carried illegally through Spain eventually to La Gomera via Tenerife. John Pierson, Paul Lloyd and Phil Smith and I (Los quatros Ingleses) spent the summer on Gomera, with a base camp in the hills at El Cedro. Paul wrote an accouint in *The Eagle*, the magazine of St John's College, Cambridge, vol 54, No 236, pp 22-26. It was culturally and botanically a fascinating place but devoid of intellectual excitement or effort, so I lost interest in bird speciation.

1953 The jellyfish action potential. *Nature* 171,400.

In the summers of 1950-1, I found many Aurelia jellyfish at Brancaster Staithe, on the Norfolk coast, and took some back to Cambridge in large jars on my motor bike to stain. I discovered that the living neurons of the nerve net were clearly visible under a phase contrast microscope that Victor Rothschild had purchased. So I spent a year building amplifiers and equipment to record from these neurons. Willy Rushton, John Pringle, but mainly the electronics technicians, showed me the way. I also visited John Baker in Oxford, where he and Kempson had developed home-made phase microscopes. I made my own phase plates from cover slips, and built a recording camera, all of which I took the following summer by train to Largs, and to the Scottish marine lab at Millport. There I camped in a bed of nettles, supposedly the Director's lawn. Aurelia was abundant, and I was able to show the first recordings of nerve impulses from a coelenterate to Sir James Gray and Victor Rothschild, who were there working on sea urchin sperm. Later they were instrumental in securing for me a Senior 1851 Fellowship and were useful support when I submitted the work for a Fellowship at St Johns. (They were really in Scotland for the salmon fishing.)

1954-1956 Nerves and muscles of medusae. *J.Exp. Biol*. 31, 594-600; etc.

One nerve impulse in the motor net was sufficient to cause a contraction of the bell, so there must be other nerve nets that carried other excitation that was not accompanied by a bell contraction. That inference was tested experimentally in these papers. I spent holidays working on Scyphozoa in various labs, extending the earlier work at Naples by Emil Bozler before he fled from the Nazis to Columbus, Ohio. Audrey helped at Millport and Naples. A lot of time was spent in the library in the Stazione Zoologica catching up with the literature on the nervous systems of the coelenterates, notably the extensive works by the Hertwig brothers on the nervous systems of Medusae (1878) and Ctenophores (1880).

I also had a job to cover my National Service, working with Jim Gordon at the Royal Aircraft Establishment at Farnborough on the design of reinforced plastic structures and cheap rocket motors for guided missiles. I had a Fellowship and rooms in Cambridge, and besides was engaged to Audrey who lived and studied in Oxford. Life was hectic, exciting and very educational.

1956. The flight of very small insects. Nature 178, 1334-1335.

As part of my work at Farnborough, I visited Prof Thom, in the Oxford University Engineering Labs. He was interested in how a biologist survived in practical research on aeroplane structures. During the conversation, he showed me some earlier work that he had done on flight at very low Reynolds numbers, and wondered if it would relate to insect flight. I took the reprint and did some calculations based on the smallest flying insects, which happen the lay their eggs inside the eggs of water beetles. They actually swim in air and use their wings under water as well as in air. For years, the paper was unique.

1956. A polarized light study of glass-fibre laminates. Brit J. Applied Physics, 6, 314-319.

At Farnborough we were using and developing new composite materials for rockets; Blue Streak and all that jazz, made from glass fibres embedded in polyester resin. How they reacted under load was known in a gross way, but not understood in detail. So, copying studies on collagen and muscle fibres, I stuck some polaroid into a microscope and examined thin test pieces of fibreglass and similar composites while they were put into compression or tension. The results were new but not ground breaking or fundamental. The fibres took the load and the resin was hardly stressed if the fibres were correctly oriented. The most loaded fibres broke first and relieved the stress very locally as the load was shifted to other fibres. In shear, the resin took the load up to a point, then the fibres caused stress concentrations in compression.

For me, the memories stuck until much later when I started to look at the construction of boats and canoes from traditional weak materials, when it became obvious that primitive man had taken measures to avoid stress concentrations.

In 1955, I accepted the offer of a job at St Andrews because the Gatty Marine Laboratory was better than Cambridge for marine invertebrates.

1957. Nervous system of the ephyra larva of Aurelia. *QJMS*, 97, 59-74.

In 1955, I accidentally found that scyphistoma polyps were budding off baby jelly fish in a tank in the reserve aquarium in the lab at

Plymouth. The ephyra larvae stained beautifully with methylene blue. The motor nerve net and the sensory nerve net were both displayed, with their meeting points at the marginal ganglia, but could not be fixed, so they were drawn and described from the living state.

1957, Co-ordination of coral polyps. *Phil. Trans. R.S*, B, 240, 495-529.

In 1955, the Royal Society gave me a grant of £100 to study a giant polychaete worm, supposedly found in coral rock in the Red Sea, of which there was a specimen about 2 metres long in the Zoology museum, Cambridge. In the summer of 1956, I put my equipment in a rucksack, found a Greek ship at Marseilles, and bought a return ticket to Port Said. I discovered that the ship was running guns into Cyprus for a war there. The Shell Oil Company at Port Said lent me a car and driver, who took me to Ghardaqa, on the coast of the Red Sea, where I stayed in the Egyptian Marine lab for 2 months.

There, quite unexpectedly, I discovered that the coral polyps were expanded at night, so every night I worked with my electric stimulator, plotting the sequences of polyp contractions when a wave of excitation spread across the colonies, using every type of coral that I could find. The coral specimens were dried and packed into the baskets in which I had purchased live pigeons from the Nile Valley for my dinners. Arriving back at Port Said, the authorities would not let me take my specimens on the ship, claiming they were antiquities. "No problem" said the Shell boss. "You go on board and wait on the far side of the ship". Soon a small boat with an outboard motor left the Shell terminal and approached. A sailor on the ship threw down a rope and my baskets of corals were heaved on board.

Because I could not find an expert on coral systematics, I later identified the corals in the basement of the Brit Mus of Nat Hist in Kensington by comparison with the original types collected in the 18th and 19th centuries. I never saw the giant Polychaete at all, but the Royal Society did not mind how I spent their £100.

1961. Discrimination of pitch by neurons of the locust nervous system. Nature 185, 623-624. and PRS, B 155, 218-231.

While writing the book with Ted Bullock, I had spent a year at the Centre for Advanced Study in Behavioral Sciences, at Palo Alto, among psychologists. While there I had the idea to train locusts to associate a sound with an electric shock *à la Pavlov*. Back at St Andrews, I organized a culture of locusts in the Gatty Marine Lab, also for student practical classes. When I recorded from the locust auditory nerve to calibrate the range of the ear, I found obvious signs that different groups of fibres responded to different pitches. There were large neurons in the

central nerve cord that also responded differentially to different pitches, so, obviously the locust was interested in the pitch of sounds. When I published this, Hans J Autrum sent me a letter saying that I must be wrong, because he had published a paper on the lack of pitch sense in Orthopteran behaviour.

1962. Postural learning by headless insects. PRS 157, 33-52.

As said above, I tried to train locusts to sound. However, the locusts ignored the sound and learned to avoid the shock by standing on one leg, so that the shock circuit could not be not be closed. So, I held the locust over a dish of salt solution while the legs hung down and got a shock when they touched the surface. The animal learned very quickly to hold up its legs at exactly the right level. Astonishingly, the learning persisted when the head was cut off.

John Pringle, critical as always, rudely suggested that it was no more than muscle rigor caused by the current. To eliminate this, I yoked together two animals, one of which had the opportunity to learn from its own movements, while the other in series with it received the same shocks no matter where it held its leg. The learning depended on the animal making its own trial and error movements, what Skinner called "operant". The passive animal never learned, instead it went limp and useless. The concept of local operant learning in the ventral cord was quite new, and it was taken up by comparative psychologists. The real significance, however, was that all insect postures, and perhaps those in many animals, are determined in the same way because the central nervous system has to continually test an unpredictable environment. Designers of walking robots later discovered for themselves the same principle.

The idea was to recur in insect vision during flight, when the insect responds to the optomotor stimulus if the environment is passively moved around it but not when the insect moves or rotates itself. Heisenberg and Wolf at Wuerzburg showed that steering in flight is controlled by the insects' brief spontaneous movements, and it quickly learns to control its direction of flight relative to the visual flow field. This rapid learning is evident if one wing is damaged. Learning is continually active in posture, walking and flight control, so experiments with clamped insects give us no idea about the mechanisms because the animals discover they are clamped and refuse to perform. This is a lesson that needs to be considered in many research themes.

1964. Multimodal interneurones in locust optic lobe. Nature 204, 499-500.

When we first stuck an extracellular electrode into a locust optic lobula, we found large spikes from neurons that responded to a variety of stimuli such as touch, sounds, body movements, as well as light or movement. This meant that the optic lobe was not only for vision. Further, it meant that the meaning of a stimulus to the animal was conditional upon coincidences of responses of neurons excited elsewhere in other modalities, and therefore conditional upon the coincidences of responses of other visual neurons. Wiersma found similar neurons in crustacea. Of course, all nervous systems work in this way, just think of Sherrington's summation, facilitation, inhibition and occlusion of excitation in the initiation of reflex arcs. So, eventually this became a constituent of the year 2006 theory of a bee's recognition of a place by the coincidences of the responses of feature detectors and cues.

Publications on Ctenophores. QJMS, 1964-1966. Symp. Zoo. Soc. 16.

While I was for a few years, from 1950 onwards, at Plymouth, Millport and Naples, intent on the organization of the nervous systems of various medusae, I noticed another group of animals, the Ctenophores, that had very elementary nervous systems and scarcely known behaviour. They are carnivorous blobs of jelly that voraciously eat plankton in all seas at all depths, so they are our competitors for food. I did not look for them, they found me. Two species occur at St Andrews in great numbers every summer.

Ctenophores are essays on the use of cilia for all kinds of purposes. In the apical organ at one end, the gravity sense comes from a few secreted grains glued to the top of four groups of cilia, which when stressed, send impulses along neurons that lie under 8 bands of cilia, by which the animal moves. The apical organ also contains light sensitive organs. A touch to the animal causes impulses in a nerve net that inhibits the cilia at other neurocilia synapses. The organization of these systems was unknown at the time The Book was written.

On the lips of its mouth, the ctenophore Beröe has huge modified cilia that contain hundreds of groups of 11 pairs of shafts in a single cell. The bending of this structure demonstrated for the first time that the mechanism of ciliary motion was the sliding of shafts relative to each other.

1965. A direct response of the crab *Carcinus* to the movement of the sun. Nature 207, 1413-1414.

David Sandeman and I had been working for some time on the movements of the crab eyestalk. One of the interesting results was the incredible sensitivity to slow movements, which peaked at an angular velocity of about 15º **per hour**. Of course, this was Earth speed. So one

day I took the apparatus outside and watched as the crab eyestalk followed the motion of the sun. Then, when the sun itself was replaced by a reflection of the sun in a mirror, the eyestalk motion reversed.

Much later, one of my students, Fred Doujak, working on a Queensland beach at night, showed that the ghost crab eyestalk follows the motion of the stars. He also showed that the light from a single star of 1st magnitude is sufficient to drive the motion of the crab eyestalk. After all, a crab eye has a larger F number than a human eye, and we see stars well enough. The significance is part of the story of navigation and homing by crabs, which at all times know their compass direction and their position relative to their burrow.

After California and the Book, back at St Andrews, I finished off the ctenophore stories. By that time, I had made a conscious decision to concentrate on all aspects of the compound eye, because the retina offered so many problems of optics, transduction, transmission of information, and control of behaviour, while the neuron anatomy of the optic lobes looked as if it might lead to an understanding of how eyes see. At the time, I was not thinking of training bees.

These early efforts defined my interests and way to progress in understanding the nervous basis of behaviour. The path forward was never defined beforehand because the discovery could not be predicted. A promising area of study was found, largely by chance; an arena for the effort was explored e.g., a little known group like the medusae, or whatever could be found in a marine laboratory. The literature was surveyed widely, guidance in techniques was found, partly by chance, and novel equipment designed and built. I tried to use modern techniques on simple invertebrate nervous systems, but the initial discovery was always unexpected and hit upon by chance. The trick was to know the literature, be continually alert, see something of interest, adopt it as one's own, then to travel, either mentally or physically, to the heart of the problem, and adapt oneself to solving it, mainly by thinking. To succeed, one must think all the time. Later, mentioning only the highlights, this method was applied to the attenuation of light guides in insect retina, the basic mechanism of range measurement and piloting by the flying bee, and the use of trained bees to analyse the mechanism of recognition of place and colour.