TODAY'S NEUROSCIENCE, TOMORROW'S HISTORY

A Video Archive Project

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Interview transcript

Edinburgh University, BSc (Hons) 1954, PhD 1960 – physiology and the construction of Henrietta

I'd always been interested in sort of zoological things, having grown up in the country. My brother did zoology, but you had to do three subjects in your first year, and then you specialised after that. And I was going to do zoology – I didn't know what I was going to do *with* it in my second year – but Ruth Fowler, who is now Bob Edwards' wife, wanted to do physiology. And I'd really hardly heard of physiology, and she wanted me to go along and see Catherine Hebb who was running the course for science students as opposed to medical students. So I went along and immediately took to the idea of doing physiology. My honours year was actually interrupted by having to have spinal fusion, which was rather new in those days, and didn't work very well. And so I, actually, more or less missed a year, and so I actually took five years rather than four years to complete my degree. And then, by that time - by the time I had finished - Catherine Hebb had moved down to the … what was then the Agricultural Research Council Institute of Animal Physiology at Babraham (Cambridgeshire), where Daly had moved from Edinburgh to be its first director. He'd actually set it all up, and so Catherine went down there and she asked me whether I would join her there and do an external Edinburgh PhD, which you were allowed to do in those days. And this was going to involve some electrophysiology, so I stayed on for the first year in Edinburgh learning some electronics and trying to build an enormous apparatus – it was called Henrietta.

This was to record … specifically to record action potentials in nerves, and it had a screen on which you could see the action potential travelling along etc, etc, and you could stimulate the nerve. You could do various things and see what the effects were, and it had all sorts of interesting components in it, called things like – in those days – 'wobulaters', and all sorts of

funny things, and most of the components were ex-War Department surplus. And all people doing electrophysiology at that time used to build their stuff from these scrap components, and there's a street in Soho that was particularly good at getting parts then. I think anyone in those days working at UC (University College London) or King's (College, London) would have spent their time getting bits, spare bits and pieces, from there.

Controversy about organophosphorous compounds

Henrietta was really rather large, and so Babraham sent our Trojan van, which went about thirty miles an hour – a top speed of thirty miles an hour – and was extremely uncomfortable, and the driver and the electronics man from Babraham trundled up to Edinburgh. Nobody had introduced them, they didn't know what each other's names were, and they spent about two days on the road not knowing each other's names. They then loaded up Henrietta, drove her back to Babraham, with luckily, all her circuit diagrams, which were exclusive to her, as so much was in those days. You built your apparatus. Only you knew what it contained.

Why I was building this was because, at that time, there was quite a lot of interest in the organophosphorous compounds, which had been developed as nerve gases in the war, and they were then being used as insecticides. And there was a lot of controversy about whether they were dangerous and whether they produced ill effects, etc, etc. And there was evidence that they produced demyelination of the nerve fibres, so the idea was that I would build this apparatus and then go down to Babraham and record action potentials, etc, etc, and see exactly what the effects of these compounds were. These organophosphorous compounds, they are … all have anti-cholinesterase effects, but some of them cause demyelination and some of them don't, and it … that is still, as far as I know, hasn't been solved. That was only half my PhD because the other half, which I did in much more close collaboration with Catherine Hebb, concerned the transport of choline acetyltransferase down nerves. And we used … we worked on goats, and we used the lesion technique where we sectioned the sciatic nerve of goats and … or ligated it, and then measured choline acetyltransferase levels at both above and below the ligature. And so this was adding to the basic knowledge about how the enzyme was being transported from the cell body - where it's made, down to the periphery where it works.

Babraham Hall, laboratories, and the care of animals

Babraham Hall belonged to the Adeane family. It was built in about 1848 on the bottom of … on top of, various much earlier buildings, and it was sort of pseudo Jacobian. And the story is that when Pevsner was writing his book on stately homes, etc, he came in the front gate, drove past the hall, and went out the back gate without stopping. It was not very genuine but

it was a big hall, and the library and the admin offices, and Daly's office, etc, were in there. The Dalys lived in the flat in the hall, and then they built a lot of temporary labs and this, of course, starting in 1948. The nation had to be rehoused after the war and so they said that they couldn't build any permanent buildings until the nation had got all its houses, and so what I worked in was called the 'initial hutted laboratories', and they are still there now in 2007.

In those days, the scientist was very much responsible for their own animals, and so when we had lesioned animals, we always used to go and visit them in the animal house, every day, ourselves. We'd all come out at weekends and do them, and if you had any kind of paralysis of anything, you made absolutely sure that there were no sort of rubs or deformity or anything like that, and so the state of the animal was paramount. And you didn't get … I mean, you didn't get good results from animals that were in pain or infected or anything like that

Institute of Animal Physiology – its first directors and scientists

The first director, as I say, was Ivan Daly - Ivan de Burgh Daly - and he took a very broad view, and what he wanted was a good scientific staff, so he recruited people who didn't have, actually, any kind of direct application to agriculture, and that's how he got a very intelligent and, initially, a very good staff. And there was no real direction … very little direction into agricultural problems. People did work on lactation, they did work on ruminants, but people also worked on things with nothing to do with agriculture. But my work on the organophosphorous compounds did have some agricultural content. And then he was succeeded by Sir John Gaddum who came from Edinburgh – pharmacologist – and he followed on very much in Daly's footsteps, of letting people do the sort of research that they were good at. He obviously encouraged people to make it applied *if* their expertise allowed them to make it applied, but he didn't insist on it, and he took an interest in the agricultural side. He used to walk about in his wellies and things like that, and then he had cancer and died, and he was succeeded by Richard Keynes, who enjoyed tweaking the tail of the headquarters in London, and not doing what they necessarily wanted to do. And once, when I wanted to go to Sweden to do something, and I asked permission to go, he sent me a little note which said, 'Go ahead. It will annoy headquarters.' Then he went off to a Chair, and in 1974 Barry Cross came from Bristol and he had a very different attitude. He wanted to do really just what the headquarters wanted him to do, and he got a knighthood for it, and interestingly, our first director didn't get a knighthood, he got a CBE. Gaddum came to the Institute, already having got his knighthood. Keynes got a CBE, but Barry got his homegrown knighthood - for compliance, I think.

Catherine Hebb was very much a major influence. She was an extremely good scientist and she also had a great feeling for people, wanting them to get on. She never wanted to steal their limelight at all. She was very encouraging but at the same time, she could be quite difficult. She had a reputation at Babraham for supporting the underdog, and so she had quite a lot of disagreements with people. And her obituaries contained lots and lots of references to the supporting the underdog. Well, Catherine Hebb set up quite a lot of contacts. I don't know the history of it really, but as a result of her contacts with various labs in the eastern block, we had several visitors working with her or with me, including Polish people, people from Yugoslavia, as it was then, and Hungarians. And one of the most active ones of the people that came was Stan Tucek from Czechoslovakia, and the interesting thing about Stan was that he was very, very staunchly anti-communist and he had a difficult time, at that period, in his institute in Prague. But Catherine managed to arrange for him to get a grant from the Wellcome after he went back to Prague, but if the authorities had known, they would have taken all the money and used it themselves. So, what happened was that we set up a system whereby we bought him things with Wellcome money and they were sent to him as a gift. And one of the difficulties about this, was that, it was okay, we could wrap up parcels of blades and things and label them gifts, but if we ordered chemicals that had to be flown out and met because they had a short half-life or something, it was very, very difficult to persuade the firms to write 'Gift of Catherine Hebb' on them and sort of not include the invoice and things like that. So that was a bit hairy.

No such thing as a good woman in science

Well, I was very lucky because working with Catherine Hebb I came, sort of, under her aura, as it were, and I have never felt a disadvantage of being a woman. I think, obviously, this is partly because not having a family, I don't have to balance work and home, etc, but also, very early on, I remember Jim Linzell saying at coffee one day. He said, 'It's more difficult for women to get educated in science than it is for men, so any woman who's made it must be jolly good.' And I've always basked in that feeling. And then we got Marthe Vogt. She came down from Edinburgh with Gaddum, and Barry Cross didn't like women and he once told my brother that there was no such thing as a good woman in science, and my brother said, 'Well, what about Marthe Vogt? She's got an FRS.' And so he said, 'Oh well, there's always an exception.' And when he had to make - and it wasn't his fault - he had to make a lot of people at Babraham redundant because the ARC changed its policy, there was a high proportion of women, and he managed to justify it. Celia Milstein, Cesar Milstein's wife, got made redundant, and so he said, 'Well, she's the wife of the Noble Laureate. She's all right,

and then Ann can be made redundant because she hasn't got a family to support,' and so it went on. Men were made redundant too, but the proportion of women was very high.

The acetylcholine system explained

Well, it had been recognised for some time that in the periphery - muscle endings and in ganglia - that acetylcholine played a role in the transmission of the nerve impulse from the end of the nerve to whatever it was - the muscle or the next ganglion and so on. But very, very little was known about the central nervous system, and so there were various ways of tackling this. Now, obviously, the thing to find out was whether acetylcholine itself was released somewhere, and whether you could measure this. You also needed to find out whether the enzyme that produced acetylcholine – this is choline acetyltransferase – was around in the place where it should be, and then when the acetylcholine had done its stuff, it had to be destroyed. Now, it was destroyed by acetylcholinesterase and so, obviously, for the system to work you also needed acetylcholinesterase, but the problem was that acetylcholinesterase was not absolutely specific. It might be there for doing something else other than hydrolysing acetylcholine, so although it was one of the easiest things to demonstrate, the fact that you got acetylcholinesterase there, didn't necessarily mean that this was a cholinergic system. And the other thing you have to remember is that, as well as possibly a cell releasing acetylcholine, there had to be cells that detected acetylcholine, and you sometimes had acetylcholinesterase in the cell that was affected. So you had to know which way things were going.

The acetylcholine system - mapping the enzymes, choline acetyltransferase (ChAT) and acetylcholinesterase (AchE)

Feldberg had long been interested in the cholinergic nervous system and he was working in Cambridge during the war when Adrian was involved in the development of these nerve gases, and because of his status as an enemy alien, Feldberg was not actually allowed to know what he was looking at. And so he used to give his samples back to Adrian labelled L1, L2, L3, etc, and Adrian asked him why he called them 'L', and he was a great opera man, Feldberg, and he said, 'Ah, well, it stands for Lohengrin, because of the recitative, Elsa - 'Ask not my place nor name, ask not from whence I came.' So, anyhow, from then on he went on working on the cholinergic nervous system and, really, on the nervous system in general, but he had done this work with Marthe Vogt, which sort of predated the Hebb and Silver [work] and that was a paper which caused puzzles.

The problem there was that there was very little, really very little difference in the various regions. I think they only found a three-fold difference between areas that were thought ought

to involve acetylcholine, and areas that didn't, and what Catherine realised was that this was a methodological problem, because the method that Feldberg and Vogt used was what we would call rate-limiting. The substrate was not available to work at a higher rate, and Catherine adopted … adapted the method, and we made our own substrate of acetyl Co A. We made it in the lab. It took about – I don't know - three days. It involved vast boilings and stirrings and all sorts of things, and the acetyl Co A was no longer rate-limiting, and we then got a big, big difference between the sensory system - virtually nothing in the optic nerve for instance - compared with other places where it was very, very high. And we did a survey of lots of various animals, and also, I think, in that paper, we had a bit of brain or two from Addenbrooke's (Hospital, Cambridge), which was easier in those days.

If you look at levels of now choline acetyltransferase, acetylcholinesterase, etc, which I did later, you've got an enormous species variation and also the … you get heavy acetylcholinesterase staining in some parts of the cerebellum where actually the choline acetyltransferase levels are very low. So you have to be very careful about interpreting things, but obviously the measurements of choline acetyltransferase give you a much better impression of the … or better results of the real picture than depending on acetylcholinesterase. And it remains, as I said, remains a conundrum why, sometimes there's a big discrepancy between one and the other, but now … now that so much more mapping has been done and one knows where projections are coming from, and where they're going to, you can have a much better idea of which cells are cholinergic, which cells are cholinoceptive, where acetylcholine is actually acting as a transmitter.

Well, in those days, we depended enormously on bioassay, and what you did was that you tried to … you took out your piece of tissue that you thought would have choline acetyltransferase in it, and you then homogenised it and you gave it substrates so it could then … if it was choline acetyltransferase, it would produce acetylcholine. Now, you had an inhibitor of cholinesterase there, so that the acetylcholine that was produced wasn't immediately hydrolysed, and then you measured the content. It was acetylcholine that you were measuring on some tissue. We mostly used the frog abdominis rectus muscle but you could also use leech and you could use various blood pressures and things, and of course, in those days we didn't know what transmitters we were dealing with. We didn't know what was in … what other things were in the homogenate, and so you used … sometimes you used three or four different systems to see if they were all giving the same indication that what you were actually working on was acetylcholine.

The acetylcholine system – investigating the nerve transport mechanism

Well, we were doing choline acetyltransferase using a lesion technique. Liliana Lubińska in the Nencki Institute in Warsaw - she was doing much the same thing looking at acetylcholinesterase, and this technique of lesioning a nerve and then watching what accumulated above the lesion and what got lost below, showed us that, in fact, there was a transport down the nerve. But the question was, you know, what the mechanism was, and there were lots of reasons for thinking it wasn't just, sort of, straight flow - straight diffusion - and then at the time, with the EM (electron microscopy) arriving, and getting some idea of the neurotubules and things, there were all sorts of theories, you know, about how it was getting there. And it got very sort of … it's become, I think, since I stopped doing it … I think it's become quite complicated because, certainly with acetylcholinesterase, there's good evidence that it goes in both directions, and you may be getting messages back from the end plates, etc … from the endings. And so we, again, were possibly adding to the body of knowledge but we were not really clear about the precise mechanisms. But we did show that, obviously, it was formed in the perikaryon and then went down to the ending where it functioned.

From electric to chemical nerve transmission

John Gaddum came down from Edinburgh about 1959, I think, and Kris Krnjevic came at about the same time. Whether Gaddum invited him to come or not, I don't know. And he had been working in Australia in the John Curtin (School), and he was joined by John Phillis, who had also come from Eccles' group, and John Phillis was at Babraham for about fifteen months. And Eccles had been very, very keen on the electric theory of transmission in the central nervous system, and he had gradually been converted to the idea that, in fact, it was transmission from cell to cell - was chemical. And they'd done a lot of good work – very important good work – in Australia, but things were still pretty controversial, and John Phillis very much enjoyed getting results at Babraham that conflicted somewhat with what had been discovered in Australia. And they were looking at the iontophoretic application of acetylcholinesterase [NB this should have been 'amino acids'] and acetylcholine, and other transmitter substances in the cortex of the cat, and they were beginning to get quite good evidence that there must be some kind of cholinergic input to these cells that were responding to the injection of acetylcholine from multi-barrel electrodes. And so, obviously, the idea was that if these cells were responding, they probably had a cholinergic input, and so the question was, 'Where was this cholinergic input coming from?' And that's where I got involved in the work of doing acetylcholinesterase staining to see if we could find what looked like suitable fibres to innervate these responding cells.

Working with Kris Krnjevic - round the clock

Kris Krnjevic's father had been a Mihailovic supporter in Yugoslavia, and so Kris as a little boy spent his time going in and out of exile depending on who was in power. And he came to Edinburgh, finally – his mother got him out of Yugoslavia and went to Switzerland – and Kris came to Edinburgh very, very young. I mean, I think he started medicine at sort of sixteen, seventeen, and he was very, very bright. And so when he'd finished doing medicine, and he'd done physiology, sort of, along the way, and he did his PhD in Edinburgh. He was a very much - or is - a very much larger-than-life character, and I had fun working with him, but he was fairly demanding and is quite a bit of a tease. And I remember when he gave … I remember him giving a lecture somewhere and saying that he'd worked with me for three years and I'd only kicked him once. And someone in the audience said, 'Obviously not hard enough!' But he was that sort of chap. No, we got on well but he was a very, very, very hard worker, and if you got a cat that was giving you lots and lots of responses, obviously, you really ought to get as much out of it as you can. And it took quite a lot of time to set the preparation up, and so we would probably not really start recording much before, sort of, six o'clock at night, and you just went on until the state of the cat deteriorated, and so we often finished about three in the morning, when you then went home. And Kris really expected you to be back by about ten in the morning to analyse the cells. And my part, was to do the acetylcholinesterase staining, but I also kept the protocol because he and John Phillis were busy turning the knobs of their machine etc, etc. And Kris complained that round about eight o'clock every night, my writing deteriorated. Well, the reason wasn't that *I* was getting tired but it was that *they* were getting tired, and I couldn't hear what they said, and I because … once you got the tissue taken out, I used to fix it in the fixative for so many hours and then it had to be washed and changed into alcohol. And depending when we finished, I often had to take this stuff home at night. And in '63 we had lots and lots of frosts and fogs and all sorts of things, and I remember taking it home to my flat and putting it beside my bed with the alcohol and things to change it to, and I had meant to wash it in water, but, in fact, the water beside my bed had frozen in the night. So it was fairly rugged sort of stuff.

Working on brain tissue with Shute and Lewis

Well, the thiocholine method is for demonstrating acetylcholinesterase, and what it really boils down to is the usual sort of thing. You have a substrate which the acetylcholinesterase works on. In this case, it would be acetylthiocholine if you were looking for acetylcholinesterase, or butyrylthiocholine if you were looking for butyrylcholinesterase. After the substrate had been split by the enzyme, you then put … you had copper there, and your thiocholine, which had been split off - became copper thiocholine. You then developed it with hydrogen sulphide and you then got a deposit of copper sulphide where we hoped … where

the enzyme had been. So you end up with a sort of browny or a black deposit, and that shows you where the enzyme was.

Shute and Lewis were in the … I think, still in the Anatomy Department. They later moved into Physiology in Cambridge, and they had been working on cholinesterase systems for quite a long time, and they used the Koelle method for staining, and they modified it. Lewis was a chemist, initially, and they modified it, and it was really that modified method that I used. And they were just looking in the brain - at various areas of the brain - but they were not, sort of, correlating it with anything very much. And so, after a bit, I did some collaborative work with them on tissue that had been lesioned. Brain lesions had been made so you could see which way these cholinesterase-containing fibres were going. We both - Lewis particularly, and I - were always very, very careful to talk about 'cholinesterase-containing'. We would never admit them to being cholinergic fibres unless we got, you know, supporting evidence, and so I did some work with them. And again, it was this business of seeing whether, if you made a lesion, there was a build-up on the side of … above the lesion, and a loss below. So that was really why I got involved with them.

Acetylcholine transmission in the central nervous system

What we were saying was that the cells that we were recording from were cholinoceptive and that they were having this innervation from the basal forebrain - and this fits with the Shute and Lewis sort of activating [system], sort of, idea. So that, although you got quite … stained cells … if you look at the cortex you saw stained cells. They were not cholinergic, they were cholinoceptive, and the source of those cholinergic fibres were way down in the base of the forebrain. The thing about the transmitters *in* the cortex is that the cholinergic system is a sort of modulatory system, and that the main transmitters are not acetylcholine. The main transmitters are the amino acids, which I was not involved with, so that what *we* found was that there were these slow, slow acetylcholine responses … acetylcholine responses are slow and different from the main transmissions. So it's a more of a modulatory system.

These cholinoceptive responses were muscarinic because, although I say not much was known about the central nervous system, the nicotinic receptors had been shown in the Renshaw cells in the spinal cord. But our responses were slow, they were muscarinic, they definitely weren't nicotinic. I mean, I think, everyone remained cautious about these things, but gradually, gradually, gradually things built up to the extent that there seemed to be a body of evidence for it - for the existence of cholinergic synapses in the CNS. Because we were interested in where these fibres we'd found were actually coming from, we were interested in the developmental side of things, and so we looked at fetal cats as well. And

one of the things that we found, which was interesting, was that there seemed to be a change in specificity during development. And in the very young fetuses, the enzymes seemed only to be butyryl- or pseudo-cholinesterase, and then cells which had had butyryl- or pseudo-cholinesterase, then seemed to develop acetylcholinesterase. But this was something that we didn't actually ever follow up.

Well, there are places where there's acetylcholinesterase, but obviously, aren't involved in transmission. Just as in the human primate placenta there's choline acetyltransferase, which is obviously not involved in transmission. And I was rather fascinated with what acetylcholinesterase might be doing, say, during development - that you get some cells which during … some neurons during development, which have acetylcholinesterase and then it disappears. And in, for instance, the cerebellum - in the Purkinje cells of the cerebellum - if you make lesions in the fibres innervating these cells, acetylcholinesterase suddenly reappears there. It's been there during development, it then disappears, but it comes back again, and this makes one wonder whether it has some kind of, sort of, metabolic effect. And there are various places where you get it and it disappears again, and particularly in development, and to me it's a bit of a mystery what it's doing.

Butyrylcholinesterase – a connection with Alzheimer's disease?

Butyrylcholinesterase, or pseudo-cholinesterase, has always been a bit of a mystery, and a lot of people just find it a nuisance. And if they're looking for acetylcholinesterase, the first thing they do is to use a specific - or near as specific as possible - inhibitor to get rid of anything that might be due to butyrylcholinesterase, and so, to some extent, you know, they have a bad press. Nobody knows what they're there for, what they're doing. We had some evidence - that's Krnjevic and me - that maybe during development there's a change in specificity, because when we were looking at embryonic development, fetal development, we found that it was butyrylcholinesterase in places which later seemed to contain acetylcholinesterase, and there are various, probably, other cholinesterases about. Now, I end, I think, in my book - my chapter on pseudocholinesterases - by saying, you know, 'What are they there for?' And then, in 2005, Giacobini had a conference in Italy, from which he produced a book, which has a great spiel – foreword – all about me and how I had, sort of, said that nobody knew what they were for, and that now, possibly, there was some indication of what they were for. And certainly, there's quite a bit of evidence in that book, which was from a meeting, that they could possibly be involved in Alzheimer's, and that inhibiting them could possibly be some kind of treatment for Alzheimer's. But again, it's all pretty unfinished.

Anticholinesterases – organophosphorous toxicity and an incident in Morocco, 1959

The compounds, which are strong anti-cholinesterases, are very potent insecticides, and that was why these compounds, which had been developed a lot during the war, possibly as nerve gases, then retained their apparent usefulness as insecticides. But they were so toxic … so many of them were so toxic and they had bad effects on people who were using them, and they … they turned up in various things - in plasticizers, for instance. TOCP - triorthocresyl phosphate was used in lipstick, and it was also used in paint and various things, and people didn't really realise how toxic they were. And then, eventually, people got worried in the wider field because of their effect on the food chain, and all these birds of prey that ate little birds, which had become contaminated, and you got a lot of loss of bird life. Although the demyelinating effect is variable from these organophosphorous compounds, between them, the actual toxic effect - the acute toxic effect - is due to the uncontrolled accumulation of acetylcholine.

But, I think that so much is probably now known, you know, about their toxicity and that they're something to be avoided, that the actual mechanisms of their action and whether they … why they're demyelinating … I mean, apart from the use by terrorists and things, and the occasional accident. In 1959, there were sudden reports in the press and on the radio about people that had been struck down by some paralysis in Morocco, and they had actually stolen aviation fluid, which contained triorthocresyl phosphate. Now, as soon as I heard it, I thought, 'That's an anti-cholinesterase thing.' And Honor Smith and Spalding from, I think, the Institute of Neurology (London), went out, and about four days later they made the diagnosis I'd already made. I think they'd made it, too, probably.

Anticholinesterases – DFP and safety precautions

DFP is diisopropylphosphorofluoridate, and it is just one of the family of these organophosphorous compounds, and we used it … it was available. Later on, we used less toxic anti-cholinesterases because we were needing to use it in various experiments. As I said, we normally used things like eserine in our bioassays, but we used DFP for … well, in the experiments I did with Alvin Burt, we used DFP to try and inhibit a whole lot of non-specific enzymes that were going to interfere with various things we were looking for. And you did have to be extraordinarily careful with it because it was volatile, so you could inhale it, and you also could absorb it through the skin. So it wasn't a very nice thing to use. In one lot of experiments when I collaborated with Ben-Ari *et al* in the MRC Neuropharmacology group, they rang me up and asked whether I would let them have some DFP, and then they asked how you diluted the DFP. And then it became clear that they had no intention of diluting it themselves. They wanted *me* to come with the DFP and do the dilutions for them,

and I was not very happy about transporting DFP from Babraham to the Addenbrooke's site where they were. So, we went very, very early one morning, and I was escorted by somebody who had various kinds of things in case we had an accident. He had oximes to inject, and he had strong sodium hydroxide to spread about the place. Anyhow, we arrived safely at Addenbrooke's about quarter past seven in the morning when there was no one else about, because the lab was a shared lab, with lots of people who could come in and out, and that's why we did it first thing. And I did the dilutions for them and we used the … injected animals, etc … and did whatever was necessary. And we had put up a notice outside saying, 'Experiment in Progress – Keep Out', but when we got outside the notice then read, 'Experiment – Suicide in Progress – Keep Out'.

In those days, general safety and biosecurity was nothing like the issue it is now, and one just had to depend on common sense and also to some extent, lack of knowledge. I mean, we knew that DFP was very nasty and you had to handle it with care, and so we did. We handled it with great care. We wore gloves, we wore plastic aprons, we had the oximes and everything ready, but there was one time when I was asked by our Radioprotection Officer if I would dispose of some chemical for him. I didn't know what it was, and it was some radioactive DFP, and it was covered in instructions about radioactivity half-life precautions etc, but not a word about having to be careful about the DFP. And he only asked me to do it because he knew that we took precautions with our DFP. PAM (pyridine-2-aldoxime methiodide) - that was one of the oximes that we used - well we would have used, we never had to use it. But it was available to reverse the effects of the inhibition, but some of the inhibition - I can't remember which - whether it was soman or sarin, or which, the effect of the anti-cholinesterase was so immediate you could *not* reverse it, but other ones you could. And I think DFP … the fact that we used, that we had oximes ready, I think suggests that DFP was, you know, reversible, if you got on with it quickly.

The Biology of Cholinesterases, 1974 - comprehensive review of function and distribution of cholinesterases in vertebrates and invertebrates

I think I started it in about '71 or so, and of course I could only do it at night and at weekends and things, and it took a long, long time. But because I'd always been interested in trying to write decent English and make things clear, I concentrated quite a lot on trying to make it readable as well as scientifically interesting, and so, I think, it did come out as quite readable. It's got about a thousand references, but it's nearly six hundred pages, I think. But part of that is because I wanted to give some kind of reference that people could look into to see where cholinesterase had been found, and I didn't want to write a great chapter saying, 'It's here, it's there, and it's everywhere else,' so I put it all into tables, and this allowed me to put in a

great number of references. So, if somebody's working on the Mongolian shrew or something, you know, they can find it in there.

This all really boiled down to what I found in the literature, what people had suggested - trying to weigh up one against the other. But unless I was pretty sure, I didn't want to make too binding a conclusion, and you did find - and I have included in the book because they were quite fun - you did find some absolutely bizarre theories. And there's one chap - and I can't remember the actual paper - but he felt that the cholinesterases varied from season to season, and that, you know, in the spring, acetylcholinesterase was working, and in the summer, butyrylcholinesterases were working. So I did put one or two things like that in, for fun.

Acetylcholinesterase in the context of transmission. That, I had to be very, very careful about because it was at this time when the ideas of cholinergic transmission were being developed, and so, again, you had to be very cautious. You had to give both sides of the story as far as possible. Because I was reading fairly widely and you came across quite a lot of conflicting evidence, it was obviously sensible to not to put too definite a point on things. I mean, some things were definitely known, they were clear, but some things were still very much up in the air, and so I tried to avoid being absolute about things.

Ethical matters – out of the lab to become Information Officer

When we started getting a lot of anti-vivisectionist activity - this was in the early 1980s – Barry Cross was very high-handed in the way he dealt with the press. He had no kind of feeling for what was going on, and he made very unfortunate mistakes. When, for instance, the local paper, which was fairly neutral on it, asked for some explanation of what we did, he just said, 'Oh well, the public is too ignorant to understand. It's not worth me wasting my time trying to explain', you know, what we did. And so, quite naturally, this really annoyed the press, and the reason why the anti-vivisectionist activity was getting so vociferous was because the government had promised that it was going to introduce a new Bill to replace … a new Act, to replace the old 1876 Act. And so the anti-vivisectionists - the animal lobby - wanted to get their say in, and they became very, very militant at that time. And, of course, Barry's remarks had made us quite a target for this sort of thing, and we had marches, and we had demonstrations, and we had all sorts of things. And because of my work with the Physiological Society, when I'd been helping … looking into the question of what was going to be licensed and what wasn't going to be licensed - things like that - Barry decided that they needed an information officer, and so he took me, finally, out of the lab and put me into

the library - made me be Information Officer and deal with all these requests and telephone calls and letters, and what have you.

Ethical editor on the *Journal of Physiology*

My specific role on the *Journal of Physiology* was as an ethical editor, and so I would always be looking at papers to see that the experiments had been humanely conducted. And one of the problems is, well - is now - was that in the old days when we worked under the 1876 Act, it was a law of the Society that we wouldn't publish anything that didn't conform with the 1876 Act, ie, something that couldn't have been done in this country. But when the 1986 Act came in, all sorts of extra things were introduced like the source of animals, the re-use of animals, the holding of animals in …the purpose-breeding of animals, etc, etc, and it was very difficult. You could no longer say that we can't publish things that couldn't have been done in this country because they infringe the law. For instance, in this country you can take oocytes from the Xenopus frog for … twice, but in the States, you can take them six times. So, you had some really, really good science and you couldn't say, 'Well, we'll reject this paper because it doesn't comply with our legislation.' So I had to make quite difficult judgements.

Sometimes, you ask for some information - they just fail to mention that they anaesthetised the animal. Well, you ask them and discover it was all perfectly humane. They just didn't put in the details. But then [with overseas papers] there are sometimes when there are details that they can't supply, particularly when they're using electric shocks, and you discover, when you ask them, that they have absolutely no idea what current they're passing, and so that sort of thing immediately gets rejected. So, it takes a bit of looking at, and then I do ring up the Home Office quite a bit if there's something I really don't like, to say, 'If, given sufficient justification, would this ever get licensed?' and they said, 'Yes, if the justification is good enough.' But sometimes they say, 'No, under absolutely no circumstances would this sort of experiment get licensed,' and so then we reject them.

Animals (Scientific Procedures) Act 1986 – animal experiments

I've been involved in the new Act since 1978 because I was on the Physiological Society Committee, and we had word that Lord Halsbury was introducing an Act into the House of Lords. But unfortunately, because of confidentiality, we didn't know about it until it was in its seventh or eighth draft, and then, at the same time, Timothy Fry was introducing a Private Members Bill into the House of Commons, and there were other, sort of, Acts or Bills that were being introduced. We looked very carefully at the various clauses and what was involved, and what effect it was going to have in physiology, and, as I said, it covers things like the housing of animals, the procuring of animals. You would no longer be able to get …

in the old days, you could get cats if a farmer had an … overrun with cats. You know, he could give them to a laboratory, and of course, very sensibly, what they [the Home Office] were worried about was the idea that people would go round in black vans and steal cats off the street. So they had to protect the source, but they went very much the other way. You had to have purpose-bred animals, your animal houses had to be kept at a temperature with a very, very tight tolerance, and there were all sorts of things that were laid down.

Now, one of the things which the Society fought very strongly for, was the question of acute experiments. Now, these are experiments, which are done, solely entirely under anaesthetic. As far as the animal is concerned, it gets an injection and that's the last thing it knows, but because they were seen to be very invasive, you know - you might open them up and you might remove this and you might do that. Initially, they were very, very strict on what was going to be allowed and what wasn't going to be allowed in acute experiments. And when you do an experiment, you sometimes get an unexpected result, and you used to be able to … under the old Act, your licence would be very broad, and it would say something like, 'Investigation of the effect of such and such on the cardiovascular system.' And so, while you were doing the experiment, you think, 'Ooh, that's useful, interesting. I'll look into that more clearly. I'll do this, I'll do that and do the other.' And as far as the animal was concerned, it made not a bit of difference to it because it was anaesthetised - fully anaesthetised - all the time. But under the new Act, they wanted absolutely every step to be written down before you ever did it, and to have it licensed. And so, if it said that you could insert three canulae into this blood vessel, that blood vessel and the other, and in the middle of your experiment you decided it would be useful to have a fourth one, theoretically, what they were saying was, you couldn't do it because you hadn't asked for permission. And so we worked very hard on getting the conditions for acute experiments to be made less, sort of, restrictive than was originally visualised. And, I mean, as far as the humanity of the experiment is concerned, it's just as humane whether you inject, you know, four drugs or two drugs. And so … and that was where we were also much helped by people like Richard Adrian. But then, also, the politics of it. The Labour party was against animal experiments but there were one or two Labour MPs - Tam Dalyell being one of them - who were very supportive of us, and we went along to the Select Committees and fed them information.

I think we won quite a bit. I think there were things that people would have liked to have seen, but I mean, we were sensible about it. We were reasonable about it and we realised that we had got to go with the, sort of, pervading feeling of the country - that animal experiments were not the ideal way of doing things, but at the minute – for many, many things – they're the best we have. And the current feeling, on, you know, reduction and

refinement and replacement, is now what people are working towards, but until you can entirely dispense with animals, what you have to do is to make sure that any animal experiment is conducted as humanely as possible, within the law.