A video interview with Professor Josephine Arendt, 17th March 2015

Professor Josephine Arendt (b 1941) is Emeritus Professor of Endocrinology in the University of Surrey. Trained as a biochemist she is a specialist on biological rhythms and has pioneered the field of chronobiology. She has researched biological rhythms and their mechanisms widely in animals and humans, including studies on jet-lag, sleep disorders in the blind, shift work, and devised techniques to measure melatonin and its metabolites. In this interview and associated material she describes her career and discusses many of these fascinating aspects of her work.

Interview conducted by Professor Tilli Tansey, for the History of Modern Biomedicine Research Group, 17th March 2015, and transcribed by Debra Gee. A longer audio only interview was also conducted, the transcript of which is available at http://www.histmodbiomed.org/file/arendt-josephine-transcript-audio-interviewpdf . The original tapes and unedited transcripts will be available to study in the Wellcome Library. Professor Arendt also contributed to the Witness Seminar on Seasonal Affective Disorder (http://www.histmodbiomed.org/witsem/vol51) and a related interview with Professor Norman Rosenthal is at http://www.histmodbiomed.org/article/sad-30. Funded by a Strategic Award to Professor Tilli Tansey from the Wellcome Trust.

1. TRAINING AND DEVELOPING AN INTEREST IN MELATONIN

I'm a biochemist by training, and the most interesting part of my life started at PhD level when I worked with Professor Merton Sandler's group on tryptophan and serotonin or 5-hydroxytryptamine, and that really has informed all the rest of my life. I learnt some very useful technology at Queen Charlotte's hospital in London. Immediately post-PhD we rather liked Switzerland, my husband and I, and we both got jobs in Geneva. I with the university and he with the Battelle Institute, and for various personal reasons I wished to look at melatonin, a hormone, which is derived from serotonin and tryptophan, so that my background knowledge was very useful at this point. And I found a group in Geneva who measured very low levels of hormones, steroid hormones, by radioimmunoassay, and in order to study this hormone, melatonin, we needed a method which was extremely sensitive like radioimmunoassay, but which had never yet been developed. The standard way of measuring melatonin at that time was to feed it to tadpoles, or to drip it on frog skin, and watch them become paler as a function of the concentration of the hormone. This was very tedious, whereas with this technique called radioimmunoassay you can process large numbers of samples rapidly.

So I was lucky. I had the right chemical background to make the appropriate reagents in order to develop this measurement technique and it measures down to 10^{-12} grams per millilitre (ml) of hormone, which is, well that's quite sensitive really, and that's the level it had to be at in order to be useful. We looked at a lot of things in Geneva days but one of the things that happened shortly after, very shortly after, publishing this measurement technique was that a psychiatrist called Lennart Wetterberg in Stockholm rang me up and said could he come and see me, he'd wanted to measure melatonin, the hormone, for a long

time and this looked like the best possibility to date. He wasn't the only person to ring up and say they wanted to measure melatonin. It turned out quite a lot of people did, particularly psychiatrists. And this is in relation partly to SAD or winter depression, because melatonin is a light dependent hormone and winter depression is a light dependent, or day length dependent, problem it appears. It's probably relative in fact. I think I get winter depression in summer when I come back to England from somewhere nice like the Mediterranean, but anyway.

Right, so lots of people were interested in measuring it [melatonin]at the time. We were interested in looking at the function of the pineal organ, which is the main source of melatonin in the body. And at that time it was thought to have a strong influence on the timing of human puberty partly because pineal tumours are associated with abnormal pubertal development. That has actually been scotched, that theory, since the 1970s which is what I'm talking about at the moment, in that the precocious and delayed puberty can be related to other, more important, phenomena probably. But nevertheless, it was associated with reproduction and it was associated with light, the pineal gland.

Okay, so we set off to try and find, look for a possible association, in humans between the pineal gland, its hormone melatonin, and the timing of human puberty. We couldn't find one unfortunately. It's never been shown conclusively that the pineal had a major influence via melatonin on human puberty but at the time we probably could have done the experiments better by being more in control of the environment. We've learnt a lot about how to measure light sensitive hormones since then and we probably didn't control the light sufficiently. However, in the meantime, we did look to see if light would suppress human melatonin as it does in animals. We only used room light intensity which is what you get in your own living room, when you turn the lights on maximum, about 500 lux usually, and it didn't significantly suppress in our very first experiment, and this is going back to '77 now. We all know now that light does suppress human melatonin if you give enough bright light, and Al Lewy was the first person to show that bright light would completely suppress it.

2. **A RADIOIMMUNOASSAY FOR MELATONIN, COLLABORATIVE LINKS AND BIOLOGICAL RHYTHMS**

I haven't really explained how the development of this radioimmunoassay happened. At the time, it's a very small molecule, melatonin, and received opinion was that it was hard, if not impossible, to actually develop a radioimmunoassay for such a molecule. But with the experience of the paediatric clinic [in Geneva] we set off and we developed the antibody which was needed and then we needed to make a radioactive form of melatonin and that was one of my major memories of this time because I did the synthesis myself using tritiated acetyl Co-A. And when I finished the synthesis I put it into our old radiation counter which had a switch to count gamma radiation, which you turned in the other direction to count beta radiation, and I hadn't noticed but it was tritiated melatonin and therefore beta radiation, the switch was on gamma. And I put my sample in, there was nothing there and I thought, "Oh, I've wasted six months of my life making nothing!" And then I realised it was on gamma and turned it over to beta radiation measurement and wow! There it was. It was the first ever radioactive melatonin and in order to use it to measure the stuff we had to count every sample 50 minutes because it wasn't very radioactive. These days you count every sample a minute or two and if you're lucky you've got a gamma radiation label of iodine usually. But in those days it was enough to get the assay up and working and to start looking at melatonin in humans, which had not been done properly except on one occasion

by a group at MIT. Harry Lynch was the first author, Richard Wurtman was one of the co-authors. They had used the bio-assay to measure melatonin in humans and they had shown a rhythm. So it did actually help in that it also gave us an idea of the sensitivity that we needed for this measurement technique.

Yes. Okay, well now that we had this measurement technique of course the world is your oyster and it was a matter of choosing the most interesting roads to follow and I'm afraid I chose several. And one of them was collaboration with psychiatrists and particularly those interested in winter depression. Yes, so we collaborated with the Swedish psychiatrist, Lennart Wetterberg, who also facilitated the development of an even better antibody for performing this radioimmunoassay through the company called KABI. He did an enormous amount of work also on his own account but we looked at a variety of things with him. One of the things that he looked at was whether light would suppress melatonin and in one person it looks as though he could show that it did, whereas I didn't use enough room light to show significant suppression of melatonin at that time. It was only later that we knew that we needed 2,000 lux from Al Lewy's famous experiment in order to suppress melatonin completely. Actually, it's quite surprising that room light didn't suppress melatonin at all because it is sufficient to change it according to the most sensitive recent techniques. And then continuing the psychiatric connection, one of my friends and collaborators in Geneva was Professor Anna Wirz-Justice. She and I found a mutual interest in rhythms at a conference, as it happens, as so often happens, and we looked at daily and particularly at seasonal rhythms in melatonin and other indoles and we were so naïve at that time that we did it on a single sample in 24 hours [at intervals during the year], and we all know now that is totally inadequate for measuring seasonal rhythm. But we did try.

And we took our data to a rhythms conference in the Italian town of Pavia. And it just luckily happened that two very important people in the American rhythms field were at that conference. One was a chap called Dan Kripke, who is still very much around and doingthings, and the other was Tom Wehr at NIH, who was very interested in rhythms and psychiatry and light, and who worked with Norman Rosenthal and with Al Lewy. So there was the meeting of the people all interested from different directions in depression, winter depression, rhythms, humans, and melatonin. At the same time, and which enhanced the importance of melatonin, we knew from David Klein's work with Bob Moore and Joan Weller that if you took the central clock out of the brain of a rat, say, it's called the suprachiasmatic nucleus. If you lesioned it you lost the rhythm of melatonin and Moore and Klein and their colleagues mapped the pathway whereby the central clock drives melatonin rhythm, this is all in rats, and light synchronises it to 24 hours in a normal light/dark cycle. And Helena Illnerova showed that as little as a minute of light at night would suppress melatonin. But the fact that this hormone's rhythm is driven by the central clock and that it can be measured in the pineal itself, in synthesis, in blood, in saliva, and it's metabolite in urine, and that all of those profiles of this hormone reflect the activity in the central clock, means that it is essentially a marker of the internal rhythmic circadian status of human beings.

And it has been enormously important in that respect throughout the whole field of rhythms research. I think we probably did a definitive experiment in the seventies. We took rats every hour during the night and we measured melatonin in their blood. We measured the melatonin enzyme, N-acetyltransferase, in their pineal glands, and we measured melatonin itself in their pineal glands. And all three sites showed highly related rhythm such that any one of them could be used as a measure of the central circadian clock activity. And later on we were to show that the melatonin metabolite in blood and in urine, and notably in urine

because it's so useful, was also a very good reflection of what is going on actually in your brain. It's a kind of window on the brain.

3. MELATONIN AND JET LAG

At this time in the, I'm talking about the eighties now, life was very busy and we'd moved back to England, setting up new labs and various other stresses. And I developed breast cancer and had a full mastectomy in 1981 and couldn't really, didn't feel right for a long time afterwards, but particularly didn't sleep very well, couldn't get to sleep in the evenings. And I thought, "Well, we know a bit about melatonin from the early work that it has some possible anti-cancer effects and we also know that it can help sleep." The first review on melatonin effect on sleep was in 1974. "I'll try some melatonin, see if it makes me feel better." And it did. So as a result of that I did a trial on a suitable number of volunteers, placebo controlled, randomised double blind with my colleague, Dr John Wright, clinician, to see if it really had phase shifting effects on people. And it did. At which point it has potential therapeutic value in illnesses, diseases, which are due to underlying disturbed circadian rhythms. And there are a rather large number of people who suffer from such things. Circadian desynchrony of shift work is one, it's very hard to treat, though. Jetlag is the most obvious one that most people know about. Your clock can't keep up with the rapid transition across several time zones. Delayed sleep phase syndrome, is another one. And probably the most important one for us, complete blindness, when if you don't have the light time cue at all then your body can't stay on the 24 hour cycle easily. So there was the therapeutic potential for circadian rhythm disturbance.

The first thing we did was a little jetlag study because that was good fun. And with the help of our head of department at the time, Professor Vincent Marks, he managed to advertise the fact that we wanted sponsorship for a jetlag study in the *Financial Times*, I believe, and we got free flights from British Caledonian Airways, we got some free hotels including the Mark Hopkins Intercontinental, which is a rather posh hotel, which is where we were intending to send our volunteers, and did indeed send our volunteers. It's a good destination to do a jetlag study because you get very definite jetlag if you're going to get it at all after flying eight time zones eastwards. I don't think our volunteers could afford to eat there but they could certainly sleep there. And no shortage of volunteers, and we had enough money to do, I think it was, 17 people. And the help of cutting edge stuff in the field because our friend and colleague, Alex Borbely in Zurich lent us actiwatches, actigraphs, which you put on your wrist and they can be geared to tell you how well you've slept, when you're awake and when you're asleep, so you could judge people's sleep. And we collected a lot of baseline data on sleep and on circadian phase and this time using the melatonin metabolite which can be done remotely.

So our volunteers were walking around carrying bags of pee and little tubes and measuring cylinders to measure the pee in and put a little bit in a tube and then wash the bottle out. And they were apparently, they had a very good time, so they told me. My mother and my husband were both volunteers, perhaps shouldn't have been, but they enjoyed it. And it was successful in significantly hastening the rate of adaptation back to UK time because we made them stay in San Francisco for two weeks so they would be adapted to San Francisco time and jetlag's always worse, nearly always worse, going east, so we did the jetlag study on the eastward leg and studied them in Guildford, in fact, when they got back, which was very convenient. And when it was published in the *BMJ* and it caused an absolute furore of people who didn't believe it and others who wanted to try it, and others who wanted to

develop it and so on and so forth. And since then there have been quite a few other jetlag studies and there's a Cochrane database review meta-analysis which says that, "Yes, it does work." You have to have the timing right, though, and we got the timing pretty much spot on as it's all based on the circadian state of the people you're treating.

4. MELATONIN, SEASONAL CYCLES AND SHEEP

Well about this, a little bit later than after developing all of this stuff in Geneva, we moved back to England and amongst other things I met up with a chap who was, this is really related to what I'm going to talk about, who knew a lot more than I did about sheep. And we got together to collaborate and obtain funds because sheep are highly photoperiodic, that is they respond to day length. It governs their seasonal cycle and because melatonin is a day length dependent hormone, or at least we wished to see if it was a day length dependent hormone, and you could show that light was important in sheep which you couldn't yet in people, so we decided to do some sheep experiments to see exactly how this hormone and the pineal gland were involved in the seasonal reproductive cycle. And heavy hints as to what it might do had come from Joe Herbert and Klaus Hoffman's labs, working in ferrets and hamsters respectively, to the effect that the pineal timed the seasonal breeding of these species, who depend on day length. If you take the pineal out they breed sort of haphazardly without a proper rhythm. And this is really important because, in the wild at any rate, the young have to be born at a season where it's propitious for their nutrition and their development.

As a result of nice, I think, data on sheep showing changes in duration of melatonin secretion with long nights and short nights, so it was long in long nights and short in short nights, and we could also shift the breeding season by giving a [winter] melatonin profile in summer to fool the sheep that it's actually winter, but thereby we can change the breeding season.

5. BRITISH ANTARCTIC SURVEY - LIGHT EXPERIMENTS

In 1984 I went to the Chronobiology Gordon Conference in Colby Sawyer College with data from our sheep experiments showing changes of the seasonal breeding cycle with melatonin and such like. It so happened that I had been asked by the British Antarctic Survey, at that time, if I would like to give the base doctor on the British bases a project, because they didn't have a great deal to do. They were dealing with very healthy young people down on the Antarctic bases. And the British Antarctic Survey knew that I was interested in light and in Antarctica the sun doesn't rise for three months in the winter and it doesn't set for three months in the summer at the British base of Halley. And so knowing that winter depression, which had been described by Al [Lewy] and Norm [Rosenthal], was apparently treatable by light treatment, that it was a seasonal effect, it seemed very likely that people spending the winter in Antarctica would suffer from winter depression in the dark days of winter. And so my idea for a project was pretty obvious. I was going to offer them light treatment for their winter depression, with controls who were not offered light treatment, of course. Not a very well-controlled experiment, let's face it, but not to worry.

And the obvious people to ask how to do this were Norm Rosenthal and Al Lewy, who were both at this conference. And so we went down to the beach one afternoon because at Gordon conferences you always get the afternoon off to go to the beach. And I said, "How much light shall I give them and how long shall I give it for? And I'm going to give them a

skeleton photo period," which is what was originally given to the first SAD patient, Herb Kern. He got three hours of extra light in the morning, three hours of extra light in the evening, and his depression remitted rapidly. Well, I couldn't tie up my Antarctic base people for three hours in the morning and three hours in the evening, and I knew anyway from experiments on animals that a skeleton photo period, a very short exposure to light at the beginning and the end of the day, can be perceived physiologically as a full photo period. So we decided to give our winter Antarctic people an hour's light in the morning, we gave them 2,000 lux of white, full spectrum light. And an hour's light in the evening. The lights were supplied by Luke Thorington of, I think it was called Full Spectrum Lighting, his company, which was very good of him. He also paid for the transport.

And the base doctor, James Broadway, did a splendid job actually making sure that these people were exposed to the light. Al and Norm didn't know how long we'd have to do it for at the time so we thought, "Well, in sheep if you want to have an effect of light you've got to do it for six weeks, so we'd better do it for six weeks in people." So we did. And we couldn't find any winter depression in Antarctica. A little low mood. So that was quite disappointing and it was, the mood stuff was done with Simon Folkard. But what we did find was that the people who got extra light compared to the people who didn't get any extra light, had a big shift in their circadian phase to an earlier time, to a more appropriate time in fact. And this is really a feature of dark winters in Antarctica, or anywhere in fact, that people tend to delay and delay until they are sleeping at an inappropriate point of their circadian phase and so they don't sleep so well. And it's quite likely that this is related to the phase delay theory, Al Lewy's phase delay theory, of winter depression. But it's not the only cause of winter depression. It can't be. All of our Antarctic people ought to have winter depression if phase delay is the reason for it, and lots of people in temperate zones with normal, what we call normal light, have phase delays. Some worse than others. If they are really bad then they have to be treated. So we knew then that skeleton light treatment would phase advance the circadian system.

This was in the same year that Chuck Czeisler's group in Harvard showed that a full photoperiod, I think it was, of bright light would shift circadian rhythms. And for years people had thought that our timekeeping, our circadian timekeeping was more to do with what are called social zeitgebers, in other words things like alarm clocks, time clues, social time cues, timing of meals, alarm clocks, time you have to go to work. But it does turn out, after all, that light is definitely our most important time cue, just like most animals. And this was all in the mid-1980s.

6. CIRCADIAN RHYTHMS IN BLIND PEOPLE; EFFECT OF MELATONIN

One of the most interesting conditions of circadian rhythm problems is completely blind people, and we know from the 1970s that they may not be able to live on the 24 hour day and this is because they, with complete blindness, including light that you don't perceive but which still gets through to your brain. In complete blindness people's internal systems do what's called free-running, that is they deviate from 24 hours, and most people deviate longer than 24 hours so that if you study the circadian rhythms of a blind person you'll get an average period, not of 24 hours, but of 24.5. This means that every day is 30 minutes different. And every so often you run completely out of phase with the normal 24 hour day and you want to sleep in the middle of the day and you wish to be awake in the middle of the night. It's like having jetlag every so often for the rest of your life and nothing you can do about it. And for some people this is really debilitating. And immediately after our jetlag

study was published I was phoned up by a blind man called Harry Kennet. “Oh, Dr Arendt,” he said, “I see that melatonin can change circadian rhythms.” He said, “I’m a blind man and I can’t live on a 24 hour day.” I’m not sure what his exact words were but essentially he self-diagnosed 24 hour circadian rhythm disorder. “Could I have some melatonin?”

Well, we weren’t just going to feed it like sweets. We said, “Will you do an experiment, please?” So we did a placebo controlled, double blind crossover on this one guy and we did a month on placebo, followed by a month on melatonin and, within four days of switching placebo to melatonin, he rang up and said, “It’s like night and day. It is like night and day. It’s wonderful. At last I can sleep at the right time.” And he was on it for the rest of his life, which was long. This happened, by the way, it was published in *The Lancet,* that one, in 1988, as a single case report. I don’t want people to forget about him, though. He was a wonderful guy. And in addition to self-diagnosing, his hobby was sculpture, as a blind man. He did animal shapes and heads and just by feel. Quite remarkable. There have been a lot of blind studies of the effect of melatonin since, notably from our group, Lewy’s group, and from quite a few others. All of them say the same thing, that you can entrain or synchronise a free-running blind person’s circadian rhythm to 24 hours with melatonin when they obviously can’t use light. And I believe Moorfield’s Eye Hospital certainly were prescribing it years ago when we were working on this. I hope they still are. But it’s not become a particularly widespread as a treatment and it should be. There is no point in giving a free-running blind man a standard hypnotic because it doesn’t do what melatonin does.

7. CIRCADIAN RHYTHM DISTURBANCES – SHIFT WORKERS

So amongst the other circadian rhythm disturbances one can think about, let me just briefly mention delayed sleep phase syndrome. This is when people have a terrible problem trying to get to sleep at night at a reasonable time and then of course they can’t get up the following day. And David Parkes at King’s and we, together, treated people with this delayed sleep phase syndrome with melatonin to advance them. It worked a treat. And it has proved, in the end, to be the one that clinicians have taken up the most, delayed sleep phase syndrome treatment, with melatonin. There’s also something called advance sleep phase syndrome but there’s very little data out there and it’s very hard to manage so we won’t go into that. Then numerically the most important for rhythm disturbance are of course shift workers. Anybody who goes from a day shift to night shift will take, if at all, a long time to adapt to that night shift and most night shift workers, the vast majority, according to meta analyses and the reviews, do not adapt to night shift. Even if you’re on permanent nightshift, your circadian rhythm doesn’t shift properly. It may shift a little bit but not the full hog for most people.

And this is because when you go home after work everything is on a normal time sequence at home. Kids have got to go to school and lunch and dinner happen at the usual time, social contacts are at the usual time and it’s very hard to completely adapt your behaviour to night shift. But we were interested in shift work in two different ways: one was if you could help nightshift workers to adapt back to day shift using melatonin to help them shift. But the other one was something that came out of the blue. Around 1995, I was asked by the medical director of an oil company if I would have a look at the circadian status of their workers because he was concerned about accidents on the nightshift which might, or might not be, due to their workers being unadapted to nightshift. And in fact most of the work we did out there was funded by the Health and Safety Executive who were very generous for many years

8. CIRCADIAN RHYTHM DISTURBANCES – GUILDFORD POLICE

Right, okay. Going back to the onshore workers, we wanted to see if we could treat shift work sleep disturbance with melatonin. Guildford police volunteered for this experiment and it was doing in collaboration with Simon Folkard, the chrono-psychologist. And we gave them timed melatonin, timed in such a way as to shift their rhythms by delay so that they could sleep better during the day. And the timing is really critical. If you give it at the wrong time of day it would advance the rhythms. If you give it at the right time of day it would delay them. So it really depends on where you are in your circadian cycle and what you want to do with your rhythms, advance them or delay them. And I think we probably were quite lucky in that we hit it right. We gave it in the early morning as they came off shift. They didn't get conflicting light, which is the problem with adapting to shift work, in the morning, conflicting light. And they did sleep better with melatonin. But the rest of the literature is rather mixed. Some people have got it to work pretty well. I think it is used in fact for its soporific qualities. It has immediate sleep-inducing, minor sleep-inducing effects, but it's not being used to its optimum capacity which is both sleep inducing and phase shifting. And then it works really well.

9. CIRCADIAN RHYTHM DISTURBANCES – OIL RIG WORKERS

Going now back again to the oil rigs, if this is relevant. Two projects were done out there and largely funded by the Health and Safety Executive, and we found that in those circumstances, when you're isolated from your family, your social life, no alcohol on the rigs, not a huge amount to do, you know, when you come off shift, the night workers working from six in the evening to six in the morning, completely adapted to the night shift. So their internal clocks had shifted by nearly 12 hours by the end of a week, in fact, and certainly by the end of two weeks. There were a few exceptions but the vast majority of people working eighteen to six out in the North Sea completely adapted to night shift, so they were sleeping fine on night shift. The trouble is when they go home they are completely unadapted to home life and they have a rotten time for the first few days back home. So whilst the recommendation would be to hasten adaption out on the rig on that schedule, it's not necessarily their preference. They would rather feel better at home and not have the first week of their time off spoilt by basically jetlag.

Some of the other schedules had very odd responses. There's a schedule which runs from midnight to midday, where they didn't adapt because the light exposure is at the wrong time for the adaptation to happen. And the rollover shifts where they, for example, would do seven nights then seven days, they spend the first week adapting to the nights and the second week trying to get back to days, so they're basically out of synchrony most of the time out there. There's another one which is seven days and seven nights. Of course, well, they're adapted to nights by the end of the seven nights but that's better than the seven nights and seven days. Sorry, I'm going to have to cough again. We have not attempted to give anybody on an oil rig any melatonin but we have attempted to use light for those people who have adapted out on the rigs to the night shift, to, when they get home, to adapt back to home time. This is hard to do because of course once people escape back home they're not all that bothered about doing experiments. But we did get some fairly positive data from giving light treatment to adapt back to home time.

10. UNIVERSITY OF SURREY, ANTIBODIES AND STOCKGRAND

In 1977 when we returned to England I quite deliberately chose to go and work at the University of Surrey because there was a very large radioimmunoassay lab there and it's a nice place to live, Guildford, and so on and so forth. And we generated more antibodies there, and at the time the antibodies were being raised on an occupational therapy unit down in Sussex in sheep and donkeys, for research and for the National Health Service, because they were used for a lot of hospital diagnostic procedures. And ultimately these antibodies were sold via a company from the University of Surrey. Well, I decided that I'd quite like to sell my own antibody via a company at that time and we had, by then we had antibodies to melatonin itself, good ones, to the melatonin metabolite, 6-sulphatoxymelatonin, and some other indoles like 5-methoxytryptophol which Debra Skene had developed. So I formed a little overnight company called Stockgrand and proceeded to sell antibodies. I didn't do it personally, I'm afraid, I engaged somebody to do it. And then people asked us to do the actual measurements, so an assayist did the actual measurements. And a lot of the collaborative work that we'd done previously developed into essentially a funds-generating system because we fed the funds that we made, the money that we made by doing assays and selling reagents, we fed that money back into research in different ways.

Last year Stockgrand had its 25th anniversary and I counted up the number of PhD students that, not necessarily all of mine but of my colleagues and myself, that we had helped, and in some cases wholly funded by Stockgrand funds and there were 18 of them. And I'm rather proud of that because it did exactly what it was intended to do, this little company. It was never large but it provided a bridge between grants and it maintained technical expertise. And technical expertise is one of the most difficult things to hang onto in a lab. There are no obvious academic posts for them but this. For example the lady that is running Stockgrand now, Dr Benita Middleton, she is an absolute whizz. She's an expert, she's a technical expert, she's also academic, she has a PhD and Stockgrand has been paying half her salary for a long time now. I should also say perhaps that it has enabled us to go to conferences, which the universities refuse to pay for. It's enabled us to have a few dinners out as well but not that many.

11. CONFERENCES AND SOCIETIES

Yes, conferences are really important. I'm very grateful to at least two English societies, the Endocrine Society or the Society for Endocrinology, I should say, and the Society for Reproduction and Fertility, for the opportunities that they have given me over our endocrinology work in sheep. Since these very august old societies were formed, we have set up something called The Clock Club in England, which is a society with no offices, it has no agendas, it has no minutes, it has no records, and it works really well because at one meeting it's decided who is going to do the next one. They get the sponsorship, get the students, and the next one is in Surrey very shortly. So that's been important. But probably, oh and the American Rhythms Society, [Society for Research on Biological Rhythms] which I've been a member of for many years, is vastly important. But the one which is closest to my heart is something with started off life founded by Professor Johannes Ariens Kappers and Dr Paul Pevet of the Amsterdam Brain Research Institute. And it was called, in 1977, the European Pineal Study Group, and I've heard it said, "Oh, it's full of European neuroanatomists" it was said. Well, it possibly was, but it was absolutely ground-breaking for

anybody working on this obscure little organ called the pineal because it put nearly 200 people in contact with each other, we met every three years, and it has now developed into the European Biological Rhythms Society of whom, well, Russell Foster was president a couple of years ago. I was president of it in the nineties when it was the European Pineal Society. And it has been really nurtured by Paul Pevet, Professor Paul Pevet from Strasbourg, who was one of the founders, and an extraordinarily energetic and enthusiastic man for keeping people together and enjoying, you know, with enthusiasm, each other's research.

12. DEVELOPING ASSAYS – A VERY PERSONAL CONTRIBUTION

Oh yes. Yes, how do you develop an assay for something that, first of all you can't buy, and secondly there is no other measurement system for it. Well, having developed the melatonin assay, I wanted a measurement for its metabolite in urine because if you can measure urinary substances you can do field studies much more easily and I wanted to do field studies all over the place, in Antarctica and so on and so forth. And in shift workers. And the major metabolite in humans is something called 6-sulphatoxymelatonin, which we usually refer to as aMT6s. And we needed to get some of it to make the antibody, to develop the radioimmunoassay, and that the only reasonable way I could think of, apart from having to do a chemical synthesis, which I didn't want to do, I just ate some melatonin. I ate quite a large amount of it, actually, I had a gram. That's a lot in our field. I felt a bit dopey for a couple of days, collected lots of pee and gave it to Chris Franey, it was who was doing it at the time, to isolate the sulphatoxymelatonin on column separation using the *sulphatoxy** group as the link.

And yes, we got our first sulphatoxymelatonin, there's still some in the lab even now. I'm talking about 1984-ish. And then it had to be labelled but that was easier because by then we knew how to iodinate melatonin which was much easier than synthesizing tritiated melatonin and it also provided a lot more radioactivity to count, so it was much more sensitive. So the sulphatoxy assay was developed with a little of my personal metabolite.

13. 'LARKS' AND 'OWLS'

I'm sure you'll have come across, most people have come across, chronobiology in everyday life because most people know whether they are early risers or, you know, late to bed. And these people have been called larks, for the early, very early risers, and the late ones have been called owls. Of course most people are somewhere in between but there are some who really, really can't go to bed at a reasonable time in the evening and there are others who get up at ridiculous times like four o'clock in the morning when personally that's the middle of my night. There's a huge difference between people and there's a lot of recent data on that from the very large databases that you get with modern technology out there. From a chronobiology point of view, from rhythms point of view, larks have early melatonin rise in the evening and owls have a later melatonin rise in the evening so there is a straight biochemical connection between the two, an early circadian rhythm, a late circadian rhythm.

The interesting thing about the early rise of melatonin in larks in the evening and the late rise of melatonin in owls is that correlates precisely to what's called the internal free-running circadian period of each individual. This means that somebody who's a lark, if put into let's say a dark room for a couple of weeks, would have a rhythm which is quite short,

shorter than 24 hours probably. And somebody who is an owl n the same situation will show a rhythm which is longer than 24 hours. With no time cues, owls have long periods, larks have short ones. Now we know from fruit flies and such like, that there are genes related to short and long free-running periods in animals. So, I am no molecular biologist but in the 1990s it was quite obvious that we needed to find out about the genes related to the circadian system. And Dr Malcolm von Schantz and Dr Simon Archer joined my group and we looked for differences in the genetic composition of owls and larks. And in fact let me take my hat off to the guys because Malcolm and Simon and in fact others from the group, set up a collaboration with the Science Museum whereby they asked people if they would like to volunteer for an owl/lark genome project.

And they questioned people about their sleep habits, whether they were early sleepers or late sleepers etc. and they took a buccal swab. And I think they got more than a thousand people to take part in this. And then what to look for because this was earlier than rapid screening of huge numbers of genes, which one can do these days. And Bambos Kyriacou in Leicester gave us some advice on what to look for. What they found is a gene called period.

There's a gene called period, there are several Per, they refer to, Per genes. And there's Per 1, there's Per 2 and there's Per 3, for the sake of argument. And Per3 was the one in which we found interesting things. *They found that there was variable number tandem repeat length polymorphism in the Per 3 gene. They refer to the alleles as 5/5, 4/5 or 4/4 and the 5/5 polymorphism was statistically significantly associated with larks and the 4/4 one with owls, the 4/5 one being more intermediate phenotypes*.* And this is the first, no it was the second, gene publication from our group. The first one was looking at another gene in which we didn't find an association. But this has gone on to become very big stuff at Surrey. At the moment they are screening for hundreds and hundreds of genes in different sleep states and different metabolic states and so on and so forth. It's all quite beyond me, I couldn't do that.

** Amended by Prof Josephine Arendt, 16/08/15*