

## **INTERVIEW WITH DR EWART (MIKE) BERTRAM AND DR KEITH MOORE, 28<sup>th</sup> OCTOBER**

PSH. It's Thursday 28 October, 2004 and I'm talking with Dr Mike Bertram and Dr Keith Moore at their residence in Toronto about the early history of the sex chromatin. What I would like to do to start with, and if I could perhaps start with you Mike, is how did you get into science in the first place?

MB. Well as an undergraduate student I was enrolled in the biology course and so I got my degree in biological science.

PSH. Which city was this?

MB. The University of Western Ontario, London, Ontario, and then when I graduated I wanted to do graduate work and there wasn't anything in the biology department that appealed to me as far as offering graduate courses. At that time there weren't all that many fields of investigation going on, and so I went down to the medical school and looked to see what different types of research was being done there, and what Dr Barr had to offer was quite appealing. He wanted somebody to work on looking at nerve cells, to see what happens after prolonged activity. He had, during the war, he was quite interested in fatigue of the officers in the Air Force.

KM. Pilots.

MB. Pilots, after many hours of being out, they'd come back really fatigued. So I was working on that project.

PSH. This must have been fairly soon after the war?

MB. Yeah.

KM . '47. '46/'47. '47 probably when you went down was it?

MB. Right, yes.

PSH. So it started off really 1948/9 or thereabouts.

MB. That's right. We made the discovery in '49.

PSH. So, before we come onto the actual discovery itself, Keith, may I ask how did you get into science and the medical field?

KM. I was like Mike. I didn't really know what I wanted. I had been in the service, I was in the Navy, and I came back and I went in the service. I didn't really know what I wanted to do. So what I did was take a combination of arts courses and science. So I ended up with a BA, which I really didn't want. My father was a clergyman and wanted me to go into the Ministry and he died, so I was taking French and German and I took creative writing, which I think

helped me in learning how to write books and so on, and so I took science and arts and did my BA and then I thought I wanted to go into zoology, so I took another year of 7 courses in zoology, and when I finished that I started to do a masters degree with Dr, what was her name?

MB. Helen Battle.

KM Helen Battle, who was a excellent scientist and I started there; then one time she said, Dr Barr is giving an interesting seminar down at the Medical College, would you like to come? So I said sure, and went down, and of course I got all fired up with this and came back and that's all I talked about in the next couple of days and I was working on Zebra fish, putting in drugs and so on, really teratology, and that turned out to be my main interest, my main interest was embryology and teratology and so she said if you are really that excited about it he is looking for people for graduate work; would you like to go down, it won't hurt my feelings? So I said well I'd like to go and talk to him anyway, so he was looking for graduate students and we had a team of what, Mike, 4 or 5 people working there at least.

PSH. What year was it you started in on this?

KM. 1950, well I started university in '46 right after the war and I went down after I got my BA in 1950 I went down to do my Masters with Dr Barr . They didn't have a PhD programme but after I finished my Masters Dr Barr developed a PhD programme. He didn't have one when Mike was there, so Mike went to Buffalo and did his PhD in neuroanatomy.

MB. Right.

KM. So he arranged this PhD programme, so I stayed with him for I guess six years and I did my PhD in four years and then I did two years post doc, and that's when we did a lot of the sex chromatin in human tissues and things like that.

PSH. Can I come back now to '48/'49 and ask, just how did it happen that you noticed this unusual structure?

MB. Well if I hadn't kept very, very careful records, probably the discovery never would have been made for maybe a number of years since, following that. And how we actually discovered it was, I was measuring this little chromocentre after prolonged activity, and there was depletion of the protein substance in the nerve cells and there was a lot of swelling, and the protein, Nissl substance, was all dissolved and this little structure was pushed away. This chromocentre.

KM. It was stimulating that, wasn't it the hypoglossal?

MB. To do all that I stimulated, activated the hypoglossal nerve.

PSH. So that went kind of backwards up the nerve to the cell body.

MB. And the reason for doing that, in sections, you had the normal control side as the left side and stimulating the right side, so under the microscope you had normal cells, supposedly, and abnormal, activated cells on the other side. So I was measuring the migration of this little structure away from its usual position and would go through a whole series of cats, because we sacrificed them after so many hours of stimulation and up to 48 hours and then 3 days, 4 days etc; so looking at all the tissue, and I had come on to the section of this one cat, drew all the measurements and then next cat the same way, and then it came to one

KM. Couldn't find it.

MB. Couldn't find it and didn't tumble to it till events

KM. Poor staining !

MB . Then we started looking up my records, because I kept track of the age and the sex and the colouration and all the rest of it. So all these things now we're recording was 'sex female'. So we began looking back at records and going through the series, every time it appeared in the animals they were female. Then when it didn't appear it was male. We were attributing it to very poor staining and other things that didn't show up. So that's basically how we made the discovery.

PSH. Can I ask, at that time what did you think this body actually was? Did you think it was anything genetic or did you think it was something physiological?

MB. Well at that time no. At that time we thought it was a little structure

KM. Called it nucleolar satellite.

MB. Yes.

KM. We thought it was, wasn't it RNA first?

MB. Well at one time

KM. We thought it was RNA first and then by Feulgen stain, wasn't that what we used, it showed up as actually DNA.

PSH. So you didn't really think you were looking at a chromosome?

MB. No.

KM. No idea

MB. Absolutely not!

KM. How long did it take, about a year to figure out or less. What it was, was actually chromatin, because they didn't seem to know much about that extra chromosome.

MB. No, there was nothing at that time

KM. And then finally we figured out that it was the extra X chromosome that was condensing and that's what it was, but it wasn't in the literature was it?

PSH. How long did it take you before you convinced yourself this was a genuine finding that it was there in females and not in males, because it must have been really quite a surprise.

MB. Oh it really was.

KM. But then Iceburger [?] followed up when I came down. You see this came out in '49 and I came down and my job was to do studies in various animals so I did

MB. He did beautiful comparative studies.

KM. I did everything, I did. I did any animal I could get at the zoo or whatever and of course it showed up in carnivores but it didn't show up in cattle, or in mice or rats or anything, it just seemed to be the carnivores mainly, I forget the details, but I did 14 different species. That's what my Masters was on, and of course at the same time you [MB?] studied human nerve cells, I forget where it was, sympathetic ganglia cells or what?

MB. Yes we did study a lot of different types of nerve cells

KM. Sure enough, was there.

MB. It was there and then what really excited us all was, went to the pathology department and pulled out some slides and looked at them, and of course pathology was well recorded whether it was female tissue or whatever, and then you could see it in

MB. Human cells, yes. And then again it was nervous tissue that we looked at and then it was Keith that did

KM. And then I did papers. I used to go down to autopsies and I found you had to get there very quickly because they soon degenerate. And so we had a colleague who, remember, what was his name?

MB. **Bob Haggar**

KM. He was the young pathologist. He would say Keith, we've got a warm one here, and I'd be down there within ten minutes.

PSH. So you had to be that quick?

KM. Well not that quick, but as soon as he got one that was really fresh I went down and suddenly we were getting beautiful pictures of human and a lot of them showing there. Before that we couldn't, and remember Margaret what was her name?

MB. Laing.

KM. Laing used to draw all these things because we couldn't photograph. I can remember standing, we had awful poor photography. I had to stand there with the technician looking down like this trying to get these things.

PSH. Because photomicrographs, they were pretty primitive in those days, weren't they?

KM. Oh yes. After a while, we got these big microscopes. You could click a button any time you want, but we had to fight to get those things.

MB. Back in those days there was little, no money for research either.

KM. So that is the way it went. He had a team working there and I stayed, as I say, and then we got into cancer, so I got a cancer fellowship for two years and I studied sex chromatin in tumours, thinking maybe the tumours were changing, but as I recall it didn't really make much difference. You could still see it. And then remember there was the one in blood too. The one they called the drumstick.

PSH. Yes

KM. That's kind of died out but again that was really sex chromatin in that - drumstick.

PSH. Tell me, what sort of a person was Dr Barr like to work with? I mean was he an enthusiast, or how would you describe him best?

KM. Well, I think he was very enthusiastic. I can tell you the stories and there are always stories. Remember, Mike, when we would make some special observation such as here it's present, I remember the adrenal medulla, gosh it just was beautiful and I could go over, and then I did the sex chromatin in babies. I developed the buccal smear test, and when we got something exciting like that he would say "we are going on a party" and then about 3 o'clock in the morning he would go and buy a great big fish and rap on one of our colleague's doors. "What do you want?". "I want to cook this fish.". Then of course they would have to get up and they would cook this fish and they would bring out all the booze, and this is the way we celebrated everything.

PSH. Was this at home or in the lab.

MB & KM. No, at home.

PSH. At home.

KM. No. We had quite a group there at one time, 15, so some of these people had gone home, gone to bed, rap on the door.

MB. Some of the wives weren't all that enchanted.

KM. Oh yeah, and there were some real characters in there too, but he was very enthusiastic; when you would come at him with something like this idea, he would write it down in his little book and then eventually we would develop it. But I can remember the first time we displayed this, at least as far as the human was. We went to a meeting in Detroit, I think it was in '52, nobody believed us that this was really of any significance.

PSH. Was that a genetics meeting, or was it a neuroscience.

KM. Triple A. American Association of Anatomy, and they didn't really believe that this was related to sex.

MB. And then there was, I think the following year, there was a demonstration against our discovery, trying to say that it wasn't so.

KM. there was a lot of doubt there for 2 or 3 years until, I think it was then when we started to culture chromosomes, and we set up a culturing thing in the early days, and I left there in '56, and then we started to get the electron microscope, all those things came in, but we started to culture the chromosomes and as you know in '59 [1956] that's when the people in Sweden showed there were only 46 chromosomes instead of 48; that's when we started to get good pictures, and so we started to culture these chromosomes and that's when we got into finding that the mentally retarded not only had, males had 3X, 4X and so on and I remember I studied all the people in a mentally retarded home in Brandon, that's after going to Winnipeg, and on all these different combinations of chromosomes, so it was clear because when you had 3 Xs you had 2 sex chromatin masses, and so on.

PSH. One of the things that interests me is, Murray Barr wasn't in any sense a geneticist by his background, was he, more a neuroscientist?

KM. Oh no.

MB Exactly.

PSH. I mean did he kind of regard this discovery as something he really wanted to pursue, or was it a sideline from his point of view?

MB. Well he was going down one road, when this was discovered he

PSH. Had to divert.

MB. I think that's the best way . . .

KM. I think he and we and more with you, but as a group, once we realised what we had we started to investigate. And I remember going over to the hospital. The medical school in those days was right adjacent to the medical school [(hospital)] so we go over and we got into the endocrinology department, and they had these Klinefelter's syndrome you see and these were almost thought of them as intersexes at that time, because you know they had small testes, female pitched voice and so on. So we took smears on them, and sure enough they were chromatin positive, as we called them.

MB. The opposite of Turner's.

KM. Yes and then we got into Turner's syndrome and we found they had no, they looked female but they had no sex chromatin and so I guess. . .

PSH. Who were the people you had contact with in the genetics field? Was Peggy Thompson already around at that point or not?

MB. Yes she was around.

KM. Well she was at Western when we trained but then Jim [Thompson] went to Edmonton and I guess you knew them pretty well in Edmonton didn't you?

MB. They went to Edmonton in 49/50?

KM. Somewhere in there.

MB. Yes.

KM. Were they there when you were there?

MB. In Edmonton?

KM. Yes.

MB. Oh yeah, but I was in the States until 1960.

KM. After Boston?

MB. Sure. I went to Edmonton in 1961.

KM. I thought you went to Wisconsin first, I see.

MB. Because I was really all in neuro and when, coming back to '49, we made the discovery just at the end of my Masters, and there was no funds or

anything available at the time, and I had already signed up and was registered and everything for my PhD in neuro so I . . .

KM. Had there been a PhD programme though you probably would have stayed on, because there was lots of work to be done.

MB. Oh yes. Would have. Exactly.

PSH. So when you say neuro, was this basic neurosciences?

MB. Right. That's what Murray was in.

KM. He taught neuro, and had a book, and it is still going. But his main interest was neuroanatomy.

PSH. May I ask then, did you then develop your neuroanatomy, or was it neuro physiology .

MB. Neuroanatomy.

PSH. Neuroanatomy. So you were never really in genetics as a speciality?

MB/KM. No.

PSH. And then went back and pursued neuroanatomy for the rest of your career.

KM. As I recall it, there really weren't genetics departments as you know, when I went to Manitoba. I think when this started during our histology lectures we used to talk a bit about the sex chromatin and that sort of thing, but there really wasn't a formal course in genetics, as I recall. As I mentioned to you earlier, when I went to Manitoba I continued this work, and that's when I did all the work with mentally defective people and so on, and I decided with the Professor of paediatrics, who was very interested he said, we should be teaching the medical students genetics, so he and I went to the Dean and of course he wasn't, where we gonna get the time. I was so keen, I had 500 or 600 hours and I said I will donate 15 hours and so we started this course, and that's when he hired Irene Uchida, who was a PhD and very good. She came and we started working this course together, and it went up and up and up, and eventually it became a department of medical genetics in the University of Manitoba. It's still there and I forget the fellow's name [John Hamerton]. He was from England, but he had a very strong department there and we worked closely with him.

PSH. And you were again primarily in anatomy?

KM. Yes, I was in the anatomy department, in fact

MB. He was Chairman



KM. I became the Chairman. I went there as a young assistant professor and went up through and became Chairman, but we carried on doing culturing and that sort of thing, but worked very closely with the clinical departments and so I think that that's about when genetics. . . it's amazing now when I think back, why genetics wasn't taught before. Everybody would throw it in. Medicine would throw in a bit, and whatever even though we were doing PhD's we were required to do medical courses. I did general pathology. I did tumour pathology because of my research . I sat in, not really sat, in on all the paediatrics and obstetrics and so on. So then we got into the intersex, which of course we figured had to be related, and that's when we got into the Turner's and the Klinefelter's, we had the first case of Klinefelter's, but Dr Barr didn't want to publish one case and we waited and of course he would go to meetings and talk about this, and somebody scooped us and came out with a paper on Klinefelter's syndrome.

PSH. I have a feeling that was Pat Jacobs from Edinburgh.

KM. Could have been.

PSH. I think so. Can I ask, after your discovery, it caused a lot of interest internationally, did you have visitors to the department or contacts with others across the world. There was one person particularly in my mind, and that was Susumo Ohno in Japan, was he directly involved?

KM. I don't think he ever came to Canada, but Dr Barr did a lot of travelling at that time, more than us because we didn't have the funds to go, but he went all over the world with this and talked about it, and he earned as you know the Kennedy award. Mike and he earned the - what was that award you got.

MB. The Goldblatt

KM. The Goldblatt

MB. Cytology.

KM. Cytology award. He doesn't know this before, but I'm a Fellow of the International Academy of Cytology and I recommended him and Dr Barr for this award and you get about a thousand bucks, not that that's important, and a big gold medal.

PSH That's nice. Most of the ones they make now aren't real gold ones are they?

KM. Oh it was real gold and I was on the committee and they were talking about Barr and I said "Look it's not Barr. Both Barr and . . ." and so they then awarded to both of them, because I think that's important historically. It is that relationship you know. In the old days when a graduate student discovered something, it was the chairman or the senior investigator got all the credit. I honestly believe it would

never would have happened if Mike hadn't said, what's going on here? He could have just said, well there's poor staining, sure they were thinking about that, but you could say, well there's something wrong with the staining here, let's work on that. And so you say, well it works good on these cats you know.

MB. And that staining was perfect for all of them.

KM. But certainly he did a lot of travelling and we went to a lot of meetings presenting this, and we wrote a lot of papers. I remember the first one we used to do, I had a piece of skin taken out here; we did the skin biopsy first and that made it easier to get specimens, so we got specimens sent to us from all over the world with these different conditions, and we would examine them and send them back and then publish papers on that and then I'd take a buccal smear and the story on that is simple, I was at a meeting

MB. Clever!

KM. And I was showing this and this one lady came up and said, I work with ducks; and I said well, I don't know whether ducks have it, but the only way to find out is to take a piece of skin and check it out and see if there's any sex chromatin in it. "Oh I couldn't do that with my poor ducks". She said can't I just scrape the mouth of the duck? I said sure you would probably get some cells and do it; oh boy (clicks fingers) that just clicked in my head. I went home and I started scraping, I got one of these metal spatulas, I scraped my own, I scraped my wife and my baby daughter who is now 50, and boy they showed up beautifully, because you just had to smear it on. Those days we used some kind of an egg fixer.

MB Egg albumen

KM. Egg albumen, then it got that you didn't even have to do that because you smeared it. Stuck it in alcohol and whatever and stained that with that, what was that blue stuff, I forget the name of the stain.

PSH. Alcian blue?

KM. And boy it would show up beautifully. So once we had that we could smear everything. I smeared, as I say, all these mental defectives, I've smeared every baby that was born in Winnipeg in a year, and that was over 4,400 and when I did that I found, I think it was 4% of those boys that I smeared had no sex chromatin, or had sex, that's right and these were the Klinefelter's. Now they were not showing anything, but when they got to be 10 or 12, when I found these I went to their doctors and said, now this child is chromatin positive which makes me think that they're probably going to be Klinefelter's, will you watch for this when they get to be 10 or 11 years old and they did. They followed up. And they said, how can I tell the patient. I said you don't. That's up to you but I wouldn't tell them.

MB. Psychological problems.

KM. You know that their testes are going to atrophy and you can start giving them testosterone, so that they can develop their male characteristics, which is what they did but those children would never be able to produce, you see. So that's how we got into that and we kept going and people would send us materials and

PSH. Did your studies overlap with John Hamerton's chromosome one?

KM. There's the name I wanted. Hamerton was the one who came to Winnipeg and came and worked in the paediatric department, I think with Irene and then they eventually formed a department, but after I had done this survey of babies, then he started to do a survey with chromosome studies and he did a huge series, and he actually proved what I'd found with my buccal smear was that Klinefelter's wasn't 1 in 20,000 as we used to think, it was one in 400 and he found that with a much bigger survey, because he could do it by taking samples and studying it for the chromosome studies, although it was much more difficult. Yes I couldn't remember his name, but I think he's probably pretty old by now.

PSH. Well it's strange, he was visiting Toronto on Tuesday, so I interviewed him. I spent a couple of hours with him.

MB. Isn't that interesting.

KM. He must be up there too isn't he?

PSH. Yes he is.

MB. What do you mean by up there?

PSH. Still here. Looking back on it now after more than 50 years, I mean, it must give both of you huge satisfaction to have been part of a major discovery like that.

MB. Yes.

KM. It certainly was an important thing, especially for Michael and for me I shouldn't call him Michael. A son of mine is Michael. But he's Mike, it's just a nickname. It meant a lot and he got a lot of recognition for this. I got recognition because of the skin tests and I developed that. The buccal smear, Barr didn't think much of that, he thought that was kind of silly and I actually did all the work.

MB. You actually had to fight to do that that.

KM. I did all the, I smeared all my kids and did all this with the technician and showed it to him [Barr] and that's when he got excited, because he could see where it was going to go, but he thought first it was crazy. We couldn't do it. Now where I got that idea, it is always interesting, in Ham's text book of histology. Do you know that one? It doesn't exist any more.

PSH. Just as a name.

KM. Well, Ham was a Professor of Histology here in Toronto.

MB. At UT

KM. He had in there, part of the preliminary stuff, take your fingernail and scrape the inside of your mouth, smear it on a slide, stain it and you can see these epithelial cells and that's where I got my idea of making the smears. So

PSH. I didn't ask you whether the smear on the duck worked OK.

KM. No, the birds didn't show it, none of the birds. But you know, rats and mice and I did everything, all the carnivores. Didn't matter whether you, I don't know whether I smeared a lion or not. I used to go to the zoo. If they had a sick animal they would kill, I remember them killing a bear.

PSH. But not especially for you?

KM. No, it was going to die anyway. So I remember trying to put the anaesthetic into the vein, and a bear, you know, their paws are so fat and everything. I was jabbing all over the place. I finally got it, but I got a lot of animals that way. I had a license. I could kill any animal in Manitoba, I had a scientific doctor's licence.

MB. How do you deal with a skunk?

PSH. Oh gosh.

KM. I had a skunk, I got them as babies, and before they started to stink I took out their scent gland, and they used to run around our house. Scare people when they came in the front door. They'd go running out. The kids loved these animals. I would take them home. I had an armadillo there and the kids would play with them for a while. The trouble was, could I kill it after I had had it as a pet. It was pretty hard. I was given a couple of lynx, I knew they would show sex chromosomes, I just couldn't draw myself to kill those beautiful lynx, so I took them out to the zoo and donated them. And we had a pet racoon and eventually we gave it to the zoo and the kids would go out and they'd call it by name, and they would give him peanuts and whatever, you know.

PSH. One of the things when I have been talking to people, I have always asked the people I have been seeing, what discovery in their careers have given them most pleasure, and almost without exception they have named something they did right at the beginning, because it was kind of what they did themselves.

MB. Highly motivated at that time.

KM. And I think that's when we got so excited, and that's when we had all these parties and that's something the average person doesn't know about. We had parties seemed to be every week.

MB. That was the challenge for Barr.

KM. Barr's sons, the oldest one became a neurosurgeon later, and he wasn't a very good student Mike, he had trouble getting through his courses but he became a good neurosurgeon. Then the second boy became a haematologist, wasn't it.

MB. Pathologist - yes

KM. Then the daughter became a librarian if I remember.

MB. Nurse.

KM. Did they have 3 boys or

MB. 3 Boys, David

KM. Oh, David became a construction contractor

PSH. Am I right that in the end Murray Barr went back into the neuroscience area, after a good long while in genetics.

MB. Well I don't think he ever left it research wise.

KM. No, he kept his book up but don't think he did research did he in neuro?

MB. No, his research prior to that was all neuro, but after the discovery

KM He was a very good teacher in neuroanatomy and he had his notes, which he used to reproduce and we tried to encourage him to write a book, but he wouldn't do it. He eventually did and it still exists and I said, look that's going to be even more successful if you get some decent illustrations. They are all line drawings but he wouldn't do it. That book still does pretty well but it would do much better. Nowadays students expect to see colour.

PSH. That's true yes.

KM. That's very expensive. Nowadays of course they want animation; I'm right in on that now,

PSH. Tell me, are there other things in this story that you feel I haven't touched on and you would like to bring out while we are here?

KM. I can't think of much more.

MB. You have covered it pretty well and Keith's book here.

KM. That covers the sex chromatin story

PSH. The book I think is very . . .

KM. And then there's all the things that came from it, every chapter, you've got chapters on Turner's syndrome and . . .

PSH. I feel very privileged that you have given me the book because I think this will be a wonderful addition to our historical library.

KM. I looked at my shelf and I said, I'm only going to keep one book of each, because we are building a new house up north and I'm going to have a smaller study and if I have one book of each and I'll tell Mary "Don't lend this book to anyone or we're in trouble".

PSH. Well Keith, if your one remaining copy goes missing you know there will be a good home which you can retrieve it from, and I am very grateful to you. I am very grateful to both of you for sparing the time and I will turn the machine off now, but it has been a privilege talking to you.

**End of tape.**