BARBARA MIGEON INTERVIEW

Session 1 - June 2, 2005

I. Family Background and Education

JENNIFER CARON: It is June 2nd, 2005, and Professor Nathaniel Comfort and I are with Dr. Barbara Migeon in her office at the Johns Hopkins University Medical School. My name is Jennifer Caron, and we're here to do her oral history interview for the Medical Genetics Project. We'd like to start at the very beginning. When and where were you born?

BARBARA MIGEON: I was born on July 31st, 1931, in Rochester, New York.

JC: Could you tell us a little about your parents?

BM: My parents were Russian immigrants, not immediate. My mother was born here and my father, I think, was born here. He was the first in his family of eight sibs, to go to college. He went to medical school and was a general practitioner. My mother hadn't gone to college. They met and married, and she was a real stay-at-home wife and mother.

JC: Do you have brothers and sisters?

BM: I'm the oldest and I have a sister who's just eleven months younger than I am and a brother who was born six years later.

JC: What plans did your family have about the education for all of you?

BM: I'm sure that my mother wanted me to have the education she didn't have, but she was really quite upset with my father, who from the time I was five years old pushed me to think about medicine as a career. She kept saying, "She'll never have a happy life. Just leave her alone, let her get married and have children." But I had a father who loved what he did. I was the firstborn, and he must have perceived something in me to push, and he did. He was very pushy. And because of him I studied medicine, no question about it.

JC: When did you start deciding that it was something that you wanted?

BM: Very late. (laughs) When I got accepted at medical school. I went to Smith College[1] and I majored in premedical science, which was a wonderful major at the time because you could take the barest minimal requirements from medical school and then be enriched in art, philosophy, and -- you know. So it was a great major. I can't really tell you that I thought I was going to be a physician, but I enjoyed the major.

I had actually gotten a job at Massachusetts General Hospital as a technician and applied to medical school. It was only when I got accepted at medical school that I had decided that that's what I was going to do. So it took a while to make that decision, considering the fact that my father, when I was five and told him that I wanted to be a nurse, he said to me, "Why work so hard to be a glorified housemaid? Just work a little harder and you'll be a doctor." (laughs)

His office and our home were together, so I knew a lot about what he did. I would

go on calls with him, sit in the car while he was seeing patients, and he'd come out and tell me everything that had gone on. I was very close to him and I enjoyed what he did, and his enthusiasm and excitement about it was very contagious.

JC: So he saw patients both at a home clinic and by home visits?

BM: Yeah. In those days, the family doctor made house calls, and he had office hours between two and four and seven and eight, and then he would go on calls to see patients. When it snowed a lot, he would take a sled in the neighborhood and put his bag on it and pull it along to make house calls. He was a wonderful family physician, and his patients really loved him. When he got sick later on – he had bad heart trouble – they would come to see him in the hospital on visiting cards just to talk to him.

JC: How did you choose Smith College?

BM: Everything was rather serendipitous. My family wanted me to go to Cornell, which was close to Rochester. They even tried to bribe me with a car. (laughs) I never really liked girls all that much, but I had gone to coed schools all my life and I found it a little…difficult, because if you were, quote, "smart," you had a hard time socially, and I had decided that maybe it would be better to go to an all girls school where I could do what I wanted to and say what I wanted to and not worry so much about looking smart. In fact, when I met to Smith, I met the smartest people I've ever met in my life, and I was not that smart. It was a good choice for me, actually. I saw women do everything, which hadn't been the case before. I'd gone to a big high school. I think there were four thousand of us. And men did everything. They were the president of student government, the head of the newspapers and the yearbook. Women never did anything. But at Smith women did everything because there was no one else to do it. (chuckles) It was very enlightening, and it was an interesting environment, because when we had lectures -- Stephen Spender[2] the poet or -- it didn't make any difference who came to talk. Even Joe McCarthy[3] came to talk one time, and we all wore red. (laughs) He shouted at us in the audience. He really lost control. But everybody went. You didn't have some date to go to, or whatever. It was something that was happening on campus and you all took advantage of it. It was a very interesting place to go to school.

JC: What kinds of things did you do? You said you had academic freedom, but you had extracurriculars, too, I'm sure.

BM: I worked *on* things. I mean, I worked on the newspaper. I was never very athletic. I played bridge. I don't know. I wasn't terribly serious. I wasn't driven in any kind of way, that was for sure. I always loved art, and I used to paint. I don't remember that very well, how I occupied my time. I like people, too, so I spent a lot of time talking, getting to know people.

JC: And when you graduated from Smith you went to Massachusetts General?

BM: No, no. I didn't go there. I had to turn down the job I had with [John B.] Stanbury[4] in the Thyroid lab. I had applied to medical school at the University of Rochester. My idea was that I'd been away for four years and I would come back home and go to medical school and live at home, and it would be less expensive. My father was never a rich man.

So I applied, and I was sort of shocked to get rejected. I had an interview with George Whipple,[5] who was a Nobel Prize winner and dean of the medical school. He

asked me what credentials I had for becoming a physician, and I pointed out that I my grades were pretty good in science, I liked science, I had been raised in a house with a physician and I knew what medicine was about. He said to me, "Why didn't I go to nursing school and reapply when I finished that?" Yes. That was pretty upsetting because I hadn't really applied anywhere else.

My father had gone to the University of Buffalo, and he said, "Why don't you apply to Buffalo?" It was late, "But why don't you do that?" At the time I can't say that I was crushed, but I was angry that I would be rejected. I had never found rejection. Life had been pretty easy. But to be told I couldn't go to medical school if I wanted to was sort of a downer, to say the least.

That's when I went out and got a job at Mass General, but I did apply to Buffalo. I didn't think much about it, and when I got accepted, I knew that was just exactly what I wanted to do so I told Stanbury I couldn't come to Boston. It was even more complex because I had a boyfriend, too, who wanted me to be in Boston, and I even decided that that isn't really what I wanted either. It was quite an easy decision, I guess. I didn't agonize over it. I just sort of knew -- that was the moment -- that I really wanted to study medicine, and this was an opportunity to do it. So that's what happened.

It was interesting because when I went to Buffalo, there were four girls in my class of seventy-five. That was a tableful for cadavers. Four of you were together, and they managed to have four of us. All the other three had been interviewed by Whipple as well and he had told them the same thing.

It's an interesting story. I went back to the University of Rochester as a visiting professor a few years ago, visiting Peter Rowley[6] there, and I told him the story. He said to me that I had several things going against me at the time. One, I was a woman. Two, I was Jewish. Three, I was short. And that Whipple had thought that – he was a very tall man – that physicians should be very tall and father figures. One of my classmates at Buffalo was Barbara Haltman, and her father was vice president of Eastman Kodak. She was quite tall, but she'd gotten the same story as I had, so I guess being a woman was probably what we had in common. But that was really my first exposure to being treated differently than what I had expected. It had been sort of easy to do what I wanted to previously.

JC: Had there been discussions and understanding of different treatment of women and men at the time when you were at Smith? Is that something that was discussed there?

BM: No. There was nothing of that sort at Smith. I mean, we had men and women faculty members and they treated us as the bright people we were. There was nothing – I didn't tell you, I got a little bored with high school. All my friends were leaving to go to college, and I still had a year to go. My father, who was always pushing me, suggested that I might try to leave high school early and go on to college earlier. He suggested I could tell the authorities that I wanted to go to medical school. I mean, he was always doing this kind of thing. And that I could use the extra time at the end.

I really had only a junior year in high school; then I went to college. So if I wanted to do that, I did that. I never felt that I had any barrier to doing what I wanted to do. The high school was complicit [and] the college didn't mind. This was the first time that I really came up against the fact that I wasn't going to be able to do what I wanted to do, at least in Rochester.

JC: Did you end up with a high school diploma?

BM: Yes. I had had enough extra credits that I the credits. I had to get a better grade in junior English in order to get credit for senior English or something like that. So, yes, I did have that.

JC: When you finished medical school at Buffalo, you came directly to Hopkins?

BM: Exactly.

II. Early Research; Marriage and Children; Working with Barton Childs

JC: Why Hopkins?

BM: Why not Hopkins? (laughs) I had spent the summer between my third and fourth year of medical school at Harvard as a clinical clerk in surgery, because I really wanted to be a surgeon. I was at Mass General Hospital, and I had a wonderful summer. I scrubbed with some of the best surgeons around. I really loved what I did, but it became quite obvious to me that I couldn't – the patients thought I was a nurse, and everybody was very uncomfortable, and there were no role models of women in surgery, so I came to grips with the fact that I probably ought to do something that was more acceptable for a woman. I decided to do pediatrics. This was recommended to me. I mean, I applied to several places – Rochester again and Boston Children's. I never thought I was going to stay in Baltimore. I just thought I was coming as – it was a good place, had a wonderful reputation. My classmates were all envious.

JC: You did your internship and your residency here?

BM: Yes.

JC: At what point did you start to move into research?

BM: Oh, you know, at Buffalo we were trained to be very self-sufficient clinically. We could practice medicine two hundred miles from the nearest hospital. You knew how to take your spinal fluids, do the assays on them, see whether your child had meningitis[7] or not. I mean, we were very self-sufficient. I always thought of myself as practicing medicine like my father, miles from a hospital, maybe even rurally. I don't know. I certainly felt that I could have done that.

I came to Hopkins, and this was a marvelous place at the moment that I was here, with a terrific faculty. Bob Cooke[8] who was head of Pediatrics, had just come, and he brought interesting people with him. There were good faculty here. We had conferences every noon.

One of the things I had to do from the time I started my internship was to give a research conference once a year. The first year all I did was to go through the records of the Harriet Lane Home,[9] which was the pediatric part of Hopkins, with children who had sarcoid.[10] It's a pulmonary disease that is rather rare in children, but it did exist and I looked at that and presented the findings that were based on case records.

I think the second year, Barton Childs[11] was on the faculty at the time, and he and I went out to test heterozygotes. We went to homes of patients with pituitary pitressin-resistant diabetes[12] insipidus. The hypothesis was that it was an X-linked disease and that the mothers who were carriers might have some manifestations, and the affected males couldn't concentrate their urine. It was always a very dilute urine. So we went out to collect urine and do specific gravity determinations[13] on urine samples, and we

found that they could concentrate their urine perfectly well. It was quite a recessive disorder. Having one mutant gene didn't affect the phenotype at all.

So I reported on that, and then by the third year I did a study of the flora[14] in the mouths and throats of patients who came into the outpatient department. My training in pediatrics was very heavily outpatient because Bob Cooke came in and revised things. It used to be that you spent half a year in the clinic and half a year on the floors with the patients [during] each of the two years. By the time I came along, it was one year here and one year there, and I ended up with three-fourths of my training in the clinic.

So I had a lot of outpatient experience, so I used it to test the flora of patients with the hypothesis that penicillin-resistant organisms would be more prevalent in people who had contact with the hospital, were born in the hospital, who had been in the hospital frequently, who had parents who were working in the hospital. Because you would get colonized with this hospital strain – and that's what we showed. I found myself a collaborator who could phage type[15] the organisms. We actually wrote a paper and it was published in the *Bulletin of the Johns Hopkins Hospital*.[16]

That's how I got started in doing research, and it was fun. It was always fun. The residents did interesting things. One of my colleagues looked at the way each of us – and it was a very independent place, very independent. You were a resident and you could do what you wanted with your patients, the patients that your interns had. We all treated bronchiolitis, which is a respiratory disease of infants. It can be very, very severe. We all had our own way of doing it. It was a viral infection, and some people didn't treat it with anything; other people treated it with this antibiotic or that. He studied the morbidity and mortality with our treatments and showed that it really didn't make much difference what we did. It would last so many days and so many would die at that point, depending –

It was very interesting and I just enjoyed it. Then it came time to try to decide what to do next. I had always been interested in endocrinology, from the time I was in medical school. We did rotations during our clinic year, and I was lucky enough to rotate with Lawson Wilkins, who was the endocrinologist here at the time. He had two fellows and the interns and residents could come [on rotation]. But one of the fellows got sick, so I was able to stay there for a month, which was a long time in my three years of training to be in a specialty clinic. And I loved it. I loved him, and I wanted to work with him. So that's what I thought I was going to do. But he had other ideas.

We're writing a book about him now, with his daughter, actually. We've been trying to do it for a while, and everybody's dying who was involved with it. (laughs) Which is unfortunate. But we're hoping to describe this fantastic man.

NATHANIEL COMFORT: Who's "we"?

BM: Well, my husband [Hopkins pediatric endocrinologist Claude J. Migeon] and I and Mel Grumbach [Emeritus Professor of Pediatrics] at the University of California San Francisco, and his daughter Betsy, who is a good friend of mine, who lives in Providence and has written the historical part of the family and his background. So we're all writing about him. It's going to be a multi kind of view of this person. He was very famous and there were lots of things written about him. Rather than rely on memories, we're going to use what was written about him at the time as other contributions. How we're going to put it together, I'm not clear, but we know what we want to do.

He was very important, and he was an interesting man because he had been in practice in Baltimore as a pediatrician. Edwards Park,[17] who was the important pediatrician here, was the one who decided that we were going to have specialties in pediatrics. He told Helen Taussig[18] she was going to be a cardiologist, for instance, and she did very well in creating the field of pediatric cardiology.

He [Park] first asked Lawson Wilkins if he would do epilepsy; [Wilkins] wasn't interested, but he made him become an endocrinologist. He did it part time for a while, while he was in practice at night and – I mean, he would teach and be a part-time person here. He had a son who was eighteen years old and got killed in an accident at Christmas. When he died, it changed [Wilkins'] life and he asked himself what he really wanted to spend the rest of his life doing. He decided he wanted to be full time, so he came in here full time.

As a consequence of the son's death, we think, he really had only male fellows. They sort of were replacements for the son in some kind of way. My husband is one of his "sons." He called them his boys. When I wanted to become one of his boys, he told me I couldn't, even though he liked me a lot and we were very fond of each other. I ended up actually even living in his house with him after he had a coronary, while Claude was away for a while, to help take care of him. He was very fond of me, but he just didn't like the idea of women examining male genitalia of young boys. That's what he said, anyway.

I couldn't argue with him. I tried. I even went to Bob Cooke and said, "Please change his mind." Bob Cooke said, "Well, why don't you work with Barton Childs? He's doing something interesting. Or become a pathologist. Endocrinology's a dead field anyway." But nothing could dissuade me, and I decided to try elsewhere. Wilkins was very good. He wrote me wonderful letters of recommendation to Harvard, one of his former fellows. And he wrote letters for me to the NIH so I got my own grant. So I went to Boston Children's Hospital to do endocrinology. I couldn't do it with him, but I did it.

JC: What happened there?

BM: What happened there was sort of – I had met my husband, who was an endocrinologist here, and he was working with Wilkins. We had not quite decided that we were going to get married. I decided I had to do something, so I went off to Boston. Then as soon as – I think even before I left, we realized that there was something going on with us and that we would probably end up together.

So I spent a year there, and then – I had a three-year fellowship from NIH – and then I requested permission to come back and work with Barton, with the extra two years. I expressed an interest in genetics, and the man at the NIH who was in charge of my fellowship program wrote back and said yes, he was giving me permission to transfer my grant from Boston back to Hopkins, but why didn't I tell him that I was getting married? (laughs)

At that point Claude thought it was not a good idea for me to become an endocrinologist in the same department that he was in, and he had good reasons for it, that I paid attention to. He had worked with a couple in the lab in Salt Lake City and it was a disaster when they were both together. People played one against the other. He was sure that I would never get credit for my work if I worked with him. He was running the lab at the time, which is where I would have gone after my first clinical year in Boston. I would have been doing research, because that's the way the fellowship program was set up.

Barton was anxious for me to come. I was his first fellow. He hadn't had anyone. He didn't have a lab, either, so it was quite interesting. (laughs) That's one of the reasons I hadn't decided to work with him before I left, because he had certainly offered me the possibility. I didn't know what a geneticist did, and I couldn't tell looking at him what he did. I mean, I knew vaguely that he thought about things a lot, but I really didn't – I was just in this kind of – the image of what a physician did was to have something to do with patients and study them in some kind of way. So it didn't sort of click with me at the time.

NC: What year is this, when you started with Barton?

BM: I came back in 1960. I started with Barton, but then we didn't know what we were going to do. (laughs) I do remember he felt very strongly – he'd thought a lot about what a person should do, so he said, "You're going to go to Homewood [the Johns Hopkins undergraduate campus] and you're going to study genetics and biochemistry again and all of this kind of thing," which I did, which I enjoyed a lot. It was such a different experience from what I'd had all the way through medical school. I mean, I was back in college again, and it was just delightful to be taking seminars. No memory involved, just enjoying the academic questions. I took Bentley Glass's[19] courses. He just died this year, actually. And Carl Swanson, who was a marvelous cytogeneticist. And made some friends there. It was a fun thing to do.

So I came back, and Barton wanted to know whether I would like to work on G6PD,[20] which is what he had done some work on. He's a klutz in the lab, so he never did lab kind of work, but he had a laboratory that one could use, and he had a technician. I said to him, "It's too biochemical." I remember that. I didn't want to do that.

At the time, it was a very exciting time in cytogenetics because chromosomes had just been discovered. I think [Joe Hin] Tjio and [Albert] Levan had looked at the first chromosomes in '56.[21] But it wasn't easy to look at them until [Paul S.] Moorehead and others made a blood method possible, where you could culture cells[22] and start looking at chromosomes for the first time.

The Moore Clinic, where Victor McKusick was working at the time,[23] had a laboratory, the first cytogenetics laboratory here, and the person who was running it was a man named Malcolm Ferguson-Smith[24]. He's actually at Cambridge now. He was from Glasgow, but he had come here just as a visiting scientist, I think, in pathology. He set up the lab, and he started working, and everybody was working with him. It was sort of an assembly line. It was quite an interesting time. We would all sit there and cut out chromosomes from photographs and label them and put them in envelopes. Then eventually you'd paste them. We were looking at all kinds of individuals for the first time, and there were lots of hypotheses about who might have an abnormal chromosome.

Especially Lawson Wilkins, he was very interested in Turner Syndrome.[25] He had suspected there was something funny with their chromosomes. You could previously look at things with Barr body determinations, sex chromatin mass[26] doing buccal [from inside the mouth] smears, and it showed that they didn't have a sex chromatin mass. When you started to look at Turner Syndrome, you found that a lot of them had forty-five chromosomes instead of forty-six, but some of them had forty-six but that one of their X's was quite abnormal. So we were learning all kinds of things. He would send over samples of blood from hermaphrodites, any people that he was seeing in the clinic.

Meanwhile, the House staff was getting very savvy, and they would send all sorts of congenital malformations over. So it was a time when everybody was discovering the chromosomal basis of disease and diseases. It was fun. I had always liked the microscope anyway, and I liked the whole thing about chromosomes.

So what happened was that Victor asked me whether I would run the lab – because Malcolm was going to leave – run the cytogenetics lab until Malcolm came back. He had some kind of feeling that Malcolm would come back. That was an interesting proposition, but Bob Cooke asked me if I would start a lab in Pediatrics and that sounded more long term. (chuckles) So I did.

I trained George Thomas, who [now] runs the cytogenetics lab [at the Kennedy Krieger Institute, established at Hopkins in 1968]. He was the first person that was put in my lab without my actually being involved in it. You could do those kind of things in those days. Someone could tell you that so-and-so was going to come to your lab to work with you. It was your boss, there was nothing you could say about it. George was a nice guy,

so I didn't mind. (chuckles) In the end, he left with my technician and he set up a lab at The Kennedy. So he was the first person I really trained myself.

JC: At this time you were working exclusively with human chromosomes?

BM: Yep. I wasn't really doing *real* research in terms of experimental research. I was really looking and reporting variants. We reported the first population variants. At the time, when people started looking, they often were looking at abnormal people, so they would come up with chromosome abnormalities and attribute them to the clinical abnormality that was present.

I was looking at my own chromosomes one time as a control. I guess I was doing some experimentation. I was looking at infants who had been X rayed or extensively looking for chromosome damage and came up with some rate of breakage. I needed controls, so I used myself as a control and found, my God, I had an abnormal chromosome. It was a thirteen that looked like a pig chromosome because it didn't have a short arm; it had been truncated at the telomere.[27] So I started getting very scared because this had been reported with chronic myelogenous leukemia,[28] with mental retardation, and things of this sort.

I went to my family, to my father and mother and sisters and brothers and found that my sister carried it, and my brother carried it, and my father had it. Then I went to his eight sibs in California. So I did a study of this variant using my own family. Claude went with me to help me draw blood.

It was quite interesting because we were able to show that haptoglobin,[29] which had been mapped to this short arm of a chromosome that I didn't have, that I was heterozygous for it, so it couldn't be there. So we took a gene off the chromosome with this study. It was fun. Studied my family. Wilma Bias[30] did all kinds of blood typing of them.

I have three Rh negative children. Claude and I are both positive. Those are the kind of things you found out with – we're so different because he's French.

JC: Knowing this, did you have any concerns when you decided to have children?

BM: Oh, by that time I had children. Within fourteen months after I was married I had my first child. Because we were not young chickens at the time, and we thought we ought to have our children while we could. I had three children in three and a half years. Barton was a little horrified. (laughs) I was on his grant at the time. The NIH came for a site visit of one of his grants -- because I was a co-investigator on his grant at this point -- and they said, "Are you contemplating having any more children?" (laughs) I felt it an inappropriate question at the moment. I had just had one, and I said, "No, not at the moment." (laughs) Two of my children had my variant and one of them didn't. I conned them into giving me blood. Bribed them. Because they were very little.

NC: Could you describe the technique? You draw blood. How do you then get the chromosomes out?

BM: You draw blood. What you want are the white cells, and these days you can separate white cells using columns or whatever. What we did at the time was just to let the blood sit for a little while until we trapped the white cells in the plasma and the red cells had settled. We'd take that and put it into a flask, closed flask, with nutrient medium. You'd have to stimulate the cells to divide because they're in the part of the cell cycle that isn't cycling, G zero cells [that is, cells in a quiescent state, not preparing to divide]. They are

unless you have leukemia, the cells from the peripheral blood, so you have to take them out of G zero and use phytohaemagglutinin, a mytogen [substance which will trigger mitosis, or cell division] from plants that was used to put in with the white cells into the culture medium. You would put it in the incubator and wait three days, take them out, spin the cells down.

Then the trick was to try to get the chromosomes out of the cells, and that's what had been discovered that made it possible to use blood. That is, if you use a hypotonic solution, which had been done by accident. The first time it was used, somebody didn't make up an isotonic solution properly and it was hypotonic. What it does is to swell the cells and you can get the chromosomes. You don't want to disturb the nuclear membrane, but you do want to get the cytoplasm away from the thing so that the nucleus can swell and you can spread the chromosomes away from each other.

You put in colchicine [a plant alkaloid] – and I forgot to mention that – near the end of the seventy-two hours, and what that does is to arrest cells in metaphase, and that's when you want to look at them. So it sort of poisons the spindle so that the chromosomes can't move to the poles to go into anaphase, and you get the metaphase chromosomes because that's when they are most characteristic. So that's what we did.

Then you had to take this solution that was swollen, metaphase cells, and put them on slides. That was a trick. That is still an art. We did all kinds of things. It was fun to watch people do it. They would put the slide on the floor and drop it from great distances in order to spread better. You use water on the slide or alcohol on the slide before you put the thing on. You could dry it either quickly or not quickly. I mean, there have been all kinds of methods used, and I'm still not sure what's the best. We used fans in order to dry fast, or a flame. Flames were things that you used to dry fast, too, until when you tried to do certain banding techniques, flaming it wasn't as good as not exposing it to flame.

It was fun to see all the techniques come about, because when we started, there was no banding. You just based all your classifications on shape, and there were twelve or so chromosomes in the middle group, Group C, that you really couldn't distinguish one from the other. You just arranged them in descending order of size and tried to pair them up the best you can with where the centromere is and how long the short arm was and the long arm. Then banding[31] came around and made it much more scientific. So it was all a very exciting time.

NC: When was banding?

BM: Oh, I don't remember what year. We went through certainly at least six, seven, or eight years without banding, and then it came [in 1969]. Caspersson,[32] he was from Sweden.

NC: Stockholm.

BM: Stockholm, yeah. Was the one who started the banding.

NC: Late sixties.

BM: Yeah. You'll have to check the dates because for me dates are – everything seems like yesterday, almost. So that's how we looked at chromosomes. We stained it with – aceto-orcein [extracted from lichens] was the first stain we used. It was awful. It would stain your fingers so that – especially if you were going out or something, you hated to have your fingers stained with this.

JC: What color?

BM: Purple. (laughs) Dark purple. The aceto-orcein we used to get from London because that was better than the stuff you could get from this country. I mean, there were all kinds of fetishes about what you needed to do in order to have the best preparation.

JC: So this was the late sixties?

BM: I think so.

JC: What *was* genetics at that time?

BM: Oh, it was pretty well developed. Barton had started a pediatric genetics group, and he had gone to the Galton Laboratory in London and met Harry Harris,[33] who was a big influence in his life. And [Lionel] Penrose,[34] who was there at the time. When he came back here, he was identified with genetics. I really don't know the flavor of genetics in terms of clinical genetics is different than it was then. You saw patients. You did family studies. You had pedigrees. I mean, from a clinical point of view, it was pretty much the way we still do it.

George Thomas had started the cytogenetics laboratory there. He was a biochemist, not a real cytogeneticist, so that was never his first love. He was doing a lot of screening at the time for protein variance and for enzyme deficiencies and for – I mean, they were doing the same kind of things that we do now. It would be interesting maybe to talk to him about how the laboratory has changed through the years.

As far as I can see, there were newer and newer more refined techniques being used, but the approaches were not different. Now you can screen for everything at the same time, and it wasn't quite that efficient, so I don't see that there were too many differences.

Barton started our Genetics Journal Club with me as a fellow, and we would meet in his home. He had a group of people who came under his influence who were not fellows. There was Mike Kaback,[35] who was working on Tay-Sachs disease,[36] Jerry Winkelstein,[37] who was a hematologist, and Jerry O'Dell, who was interested in bilirubin.[38] I mean, there was a whole group of faculty in our department who were working on real genetic problems, even though they weren't geneticists.

That was Barton's idea. He always thought that genetics should be an outgoing kind of thing. You don't bring patients in. So he never had a big clinic. There *was* a genetics clinic, but it wasn't a big encompassing one. He always felt that we should go out and help people in their clinics rather than bring everybody in to a genetics clinic. Because genetics was bigger than that. You understand what I mean.

JC: This was a different approach than Victor McKusick's.

BM: Yes. Victor was a clinic builder. It was interesting. I feel fortunate to have grown up with being influenced by the two of them. I used to go to the Moore Clinic conferences as well when I was working over there in cytogenetics. It sometimes disturbed me. He [McKusick] would bring patients in to be sort of *studied*, but he wasn't taking care of them perhaps. He had endocrine patients coming in with goiters and things. The endocrinologists would get very upset because their patients would be called in to a clinic, and they would ask the endocrinologist, "Why am I going here?" But, you know, people go where someone summons them. He was collecting these people but not really taking care of them. I thought that was not good. That reinforced the idea that you really want to leave

people where they are, and with people who can take care of them. And give those people the advice about how to study the individuals, if they need to do that, or collaborate with them, or do something like that, rather than the other.

He did bring in a lot of interesting patients, and you always had interesting people to see. I could see it from Victor's point of view, and I could certainly see it from Barton's point of view, and I could see it from the endocrinologists' point of view. They were tugging for patients, which still goes on, as you know. (laughs) People don't want their patients to be put into someone else's study without being a part of it.

JC: So Victor McKusick was in the Department of Medicine.

BM: Mm-hmm.

JC: And Barton Childs was in Pediatrics. How do these departments fit together in the medical school?

BM: How do medicine and pediatrics fit together? Well, it wasn't any different than it was anywhere else I'd ever been. I'd been at Mass General and I'd been in Buffalo. That's my experience. They were pretty much separate departments. There wasn't a terrible amount of interaction between them, and there still isn't an enormous amount of interaction between them. Mike Weisfeld [chair of Medicine at Hopkins] runs his show, but I don't know how much interaction there is between what George Dover [chair of Pediatrics] does and what he does.

My husband is actually going out to San Diego for the endocrine meetings and he's giving a talk about congenital adrenal hyperplasia,[39] which is a steroid disorder, an inborn error of steroid metabolism. He's being asked when do you give up your patients to medicine? Because pediatricians often see these patients, and then when do you turn them over to an adult physician? He would say, for this disease, it's usually bad when you do because the philosophies are so different in medicine and pediatrics. Certain disorders, it makes no difference. People tend to want to get rid of diabetics as fast as they can because they're very painful to take care of.

Pediatricians care whether you comply. When they give you an appointment, they call you up a week beforehand to remind you of that appointment, and if you don't make it, they call you to bug you about it. But if you go to an internist, as you and I all do, they don't care whether you make an appointment or don't make an appointment, and nobody's going to call you up and ask you why you didn't show up. It's an entire different philosophy.

So if you have something where it's pretty critical, for example, not allowing a female to masculinize too much, then you might want to keep her under control yourself because you know you can do it better than to send her on. I think there's different philosophies. There's even trouble about when you part with patients, even if they're – he's got some patients that are forty years old and they're still being seen in pediatrics.

III. The Lyon Hypothesis and X Inactivation

JC: I think it's going back a little chronologically, but I was wondering if you could tell us about Mary Lyon.

BM: What would you like to know about Mary Lyon?[40]

JC: When did you first interact with her or her work?

BM: Well, OK. Mary Lyon – and there is a wonderful – have you seen Ben Lewin's web site, which is called Ergito?[41] I think you should see it. It's in Latin and I wouldn't know. Anyway, he's asked about a hundred scientists to talk about their discoveries and how they made the discovery. She's written a wonderful piece about how she discovered it, so my knowledge is not first hand. I can tell you that Mary Lyon is a very private person, and everybody will tell you that it's hard to have a conversation. She's very shy. You ask a question and it may take a long time for her to respond to it. When she does, it's very carefully considered, and it's always marvelous.

I've never discussed any of this with her, of how she discovered it, but she's written about it so much and she writes so beautifully that you have no problem in knowing about these things. She was at Harwell, sort of the British equivalent of the NIH.[42] She was a mouse geneticist, and she tells the story that she had looked at pigment phenotypes in mice and had made the observation that when the gene for the mutation that caused the abnormal pigment was on the X chromosome that the male was completely mutant and had a completely homogeneous absence of pigment, but the female, who had a normal gene in the mutant, you would have expected a blend of the colors. You didn't get a blend. You got a salt and pepper or a bigger patchwork kind of distribution of the pigment.

That set her to thinking, how would you do that? I think she sort of thought almost of the possibility that only one X was being expressed in each patch of pigment. But she couldn't say that. It wasn't clear in her head until a few things happened, and they had to happen. One was, she had to know about the XO mouse, which was discovered in mice by Liane Russell, who reported it.[43] So she knew that an XO female was viable, that one X chromosome could be enough. They were even fertile, so it wasn't like Turner Syndrome in humans. So you could even be a fertile female with one X chromosome.

Then Susu Ohno, who was a Japanese geneticist in California, at the City of Hope,[44] had observed – and she knew about the Barr body,[45] because that had come pretty early. He [Ohno] had made observations in the rat, somatic cells, that there were differences between males and females in terms of condensed chromosomes that he called X chromosomes, that he could see a condensed X chromosome in females and he didn't see it in the male. So he wrote a paper and said this was the condensation of the X chromosome. He never called it an inactive X chromosome.

At the time, even though it seems so obvious to us, people didn't know what the Barr body was. They thought that maybe it was the intersection of the two X's in the female, that somehow or other they paired together and made a body, which you didn't see in the male. So they were not quite sure what it was. They didn't think of it as a condensed X chromosome.

He was the one to show that there *was* a condensed chromosome. He followed it from prophase to metaphase, and he could show that in interphase it was more condensed, and then it got to be less condensed as it headed towards metaphase. Or it became obvious. I'm never quite sure at how condensed the chromosomes really are at metaphase, the inactive X – I don't know. Certainly, all the other chromosomes that are spread out during interphase become condensed, but the inactive X may stay the same throughout the cell cycle. I'm not quite sure about that.

Anyway, he showed it and reported it. She said in this web site article that she needed to see that [observation], and that's what really told her. I of course asked Susu many years later why he didn't say it was an inactive X chromosome. He said to me that he did in his discussion, and he was made to remove it because it was considered hand waving and he didn't have the evidence for it, so he had to take it out.

My idea of how things happen is that there are ideas in the air and it comes to lots of people at the same time. We have enough knowledge that leads a lot of people to the

same idea. So he certainly had it, and Liane Russell had it. Some people call it [the Lyon hypothesis] the Lyon-Russell hypothesis because she wrote about this as a possibility of a single active X, but it was buried. She wrote about it, it was published in *Science* at about the same time. I think Mary Lyon's was April and hers was July.

JC: Nineteen sixty-one?

BM: Yeah. And in *Nature.* But if you read her paper, which is a *long* paper for *Science*, nine pages. I mean, it's really very dense. It's called "Sex Chromosomes in Mice," or something like that.[46] To find her hypothesis, it's just one paragraph in this long paper. And it's there. It's quite clear that she understood the same thing. But it was Mary Lyon's brilliant exposition of this hypothesis that made it so compelling. I read it every year as I talk to students. I go to Sarah Lawrence [College] and talk to the genetics students. I think it's a lot of fun to read that, because I tell them that it's not what you do alone, but it's how you package it that's very important in whether your science is going to be remembered or not.

That's what clearly happened to Liane Russell, who was a very good scientist. I mean, here she described the XO mouse, she talked about X autosome translocations. She did much more experimentally than Mary Lyon ever did, in *this* field. Mary Lyon has done very nice experiments with the T cell mutations in mice. I don't mean – what is it called? They're the T mutations. They have to do with the tail length and with the meiotic distortion in terms of sex ratio. She's done a lot of experiments, but she hasn't done much with this field.

JC: How does the Lyon hypothesis relate to G6PD?

BM: OK. (chuckles) How does it relate to G6PD? Well, when Mary Lyon put together her hypothesis, she essentially suggested the way to test it. She said there was this thing of active X. She said that once inactivation occurred, it was fixed, so that all the progeny of the cells would have the same active X, which is why you would get patch sizes of pigment that were very large because of cell migration during embryonic development. And you have clonality there.

It was Barton Childs with his second fellow, who was Ronald Davidson,[47] who is Canadian, who came to work with him, who decided they would test the hypothesis. Now, at the time, there weren't too many variants known on the X chromosome, but there was a very handy variant for the enzyme G6PD. About forty percent of black females have two different alleles at the G6PD locus. Most Caucasians have only a single G6PD allele, except when you get to the malarial belt, where you get a lot of mutations because it is protective.

In this country, most people have G6PD B, and in the blacks, there's a variant called A and it can be A(+) or A(-). Those are just sequential mutations affecting the migration. The A(-) leads to lower activity of the enzyme, and it's associated with hemolytic anemia[48] in blacks. But the A(+) variant is just a polymorphic variant. Both of them are present in about twelve percent of blacks.

Anyway, the heterozygotes, there are forty percent of blacks that are AB, so it's quite frequent, which is very useful. The marker can be discerned at the cellular level. Now it's much easier, but at that time, what you did was starch gel electrophoresis[49] and you could separate the A variant from the B variant. The A(-) was only visible in blood cells because you lose the nucleus, so it's an unstable enzyme and you wouldn't see it there. But if you use fibroblasts[50] from an individual who was A(-), it wouldn't make any difference. You would see the two populations.

One migrates slower than the other, so what they decided they would do was to find heterozygotes for G6PD, these black females, grow up their skin cells and then clone them. Get single cells and let them grow into a population that you could do a starch gel electrophoresis on and see what the phenotype of the clone was. Now, Mary Lyon would predict that some clones would be A and some clones would be B, and there would be none that would be AB since one X is marked with A, and the other X is marked with B.

And that's precisely what they found, that the uncloned cells had both A and B, and the clones had either A or B. So it was the first proof of the hypothesis that it had validity because it said not only is there one X functioning in each cell but that it's clonally propagated so that all the cells in the clone have the same X.

At that time we didn't know the structure of G6PD because it becomes quite obvious it's a dimer. If both A and B are being made in the same cell, you should get the heterodimer,[51] which would be a third enzyme that migrates in between. You don't see it, so you'd know immediately just by assaying the enzyme that they got to be in different cells, because if they were in the same cells, you'd have a third band, which was the heterodimer, between them.

We did find heterodimers, and we found them in germ cells where the two X's are functional, and there you get the three bands. We find them in triploid individuals, humans who have sixty-nine chromosomes but have an XXY or an XXX karyotype. They have heterodimers because they have two active X's.

It's been a marker for two active X's ever since. In other words, if I would try – and at the time we did some of the first studies to see if you could reactivate an X and how stable that inactivation was. We'd start with a clone, or start with a population. I've transformed them with SV40.[52] I've done all kinds of things to cells, treated them with chemicals and whatever, and shown how stable this phenotype is. What we were looking for was the appearance of heterodimers that would tell us we absolutely got both X's expressed in the same cell. So it's been a very good marker for the activity state of the X chromosome in any kind of cell.

JC: How did you end up working on X inactivation?

BM: I started working on X chromosomes from a cytogenetics point of view. I had said that it was too biochemical to do G6PD, so Ron Davidson did these critical experiments with Barton. But as I was in the lab, I realized that it was an interesting problem and that I needed to get more biochemical, that I could look at chromosomes, but if you really wanted to know – so we started making hybrid cells. That was the thing to do. You could hybridize cells and we could try to see whether we could induce X inactivation in hybrids. We couldn't. So I started to get really involved. I was using these G6PD markers, as a lot of people did at the time. Then other ones came along. So I just sort of drifted into studying the process.

Meanwhile, George [Thomas] was involved in the clinical lab, and I didn't need to be involved clinically at all. I had the freedom because nobody cared what I did (chuckles) of doing just what I wanted to as long as I could support myself.

IV. Grant Support; Mentoring Students; Career Management

JC: And how were you supporting yourself?

BM: Well, initially I was on Barton's grant, and then I applied for my own in '71 and got it. Then continued with the same [NIH] grant, just renewing it every five years. It was a wonderful time, when you could get a five-year grant and do a renewal just once every five

years and sit there and think about what you wanted to do for the next five years, which was always a good exercise. Submit your grant, it would get funded, no problem. You just continued to go on. I've always supported myself that way.

JC: So you kept that grant from 1971 to 2003.

BM: To 2003, yeah. There was one point where they started wanting to have more grants. My grant was always a Program Project. I'd have three or four different projects I was going to work on. Now they don't like that. They really want you to focus on one problem and then write another grant for the next problem, and so forth. At that time I could do that, but there was one point where I was encouraged by the people at NIH to apply for a grant. I guess something opened up and I didn't have it on the original and I could apply for a separate one. So I got one for a five-year period of time. When I tried to renew it, that didn't go. I can't remember what quite happened, but it didn't continue, so I just went on with the one I had and had that until, yeah, 2003.

NC: Which institute?

BM: NICHD, the Child Health and Human Development Institute. It was good. Those were the good days.

JC: Technically, you were on soft money for quite a while then.

BM: I was always on soft money. There was never – I mean, we're so different from the basic science department, where I think fifty percent of your money is hard if you're teaching. But we don't have that. I was always ninety percent on grant or ninety-five. I started the Ph.D. program in human genetics, and in that role as director, I got twenty percent of my salary from the medical school. So then I could go to eighty percent. But it was always like that. Your department head didn't want to invest anything in you, he didn't have to, and you just did that.

It wasn't such a hardship, as I told you, because you only had to write every five years, and you could put your salary on it. There weren't too many caps [on how much salary could be requested], and certainly the caps didn't hurt the salary level at Hopkins. Then it started getting more difficult, the last few grants. The last grant I wrote became difficult, and I was told that I was too advanced in stature to have eighty percent of my salary coming from the grant and – whatever. Hopkins has always had the philosophy that if you spend eighty percent of your time doing research, it should be supported by a research grant. That's what it was. It's always been soft money.

It wasn't terribly anxiety-producing for a while. It's only with the lower level of funds available and the greater influx of people asking for that money. We have to have more scientists asking for money, don't we, than we did then? I used to go to meetings and there were only two hundred people total and now there are thousands. You had to go to meetings because if you didn't, you wouldn't know what would happen for a year because it took a year to publish a paper. You went to meetings to hear what's going on. Now you don't do that because people won't even tell you what they know very well. Did you go to that conference on epigenetics[53] yesterday?

NC: I wasn't able to go. Carol [Greider][54] went.

BM: Well, it was a very good conference. I enjoyed it a lot. But there was one speaker who, when asked about something he'd presented and about how he did it, he says, "I'll

tell you later." That was the answer to his question. I can't *believe* that you can get away with that kind of behavior. Why talk about it if you can't really answer people's questions about it? But it was a very good meeting nonetheless. It was good. I'm talking too much.

JC: That's the point. You mentioned founding the Ph.D. program in human genetics. I'm wondering about that in terms of what the process of founding it was like, and I'm also wondering about human genetics versus medical genetics.

BM: Oh. Well, it's another Barton Childs kind of outlook. Victor [McKusick]'s always been interested in medical genetics. Barton has always seen genetics as much broader than that. He's interested in genetic variation responsible for normal behavior, not just disease. When we talk about human genetics, it includes medical genetics, but it's broader than that because it talks about all the components, not just the mutations that are responsible for disease. So we called it human genetics.

I really love that program, and it's quite interesting that Howard Hughes [Medical Institute][55] is now proposing to start programs like ours. We're applying for it, but we have the problem that we have the program. (laughs) It was very obvious to us. It was the first program, aside from immunology, that came out of clinical departments and was governed by a board rather than a basic science department, so we had a board for the program. That was the governing body of it. But it emanated from clinical departments, and it was very difficult at the time to get a graduate board to pay attention to programs that would emanate from clinical departments. For that reason, we got [the] Homewood biology [department] involved. Phil Hartman[56] was the most marvelous person, absolutely marvelous. He agreed to be co-director of the program with me. I was completely a novice about graduate education and how to do things, but he just was a great guide.

The idea was, we all wanted to have graduate students, and Hopkins was such, and still is, where the BCMB [Biochemistry, Cell & Molecular Biology] program wouldn't let people come to preceptors in clinical departments, so we didn't have access to students from that program. We didn't have graduate programs anywhere else, like pathology, and whatever else. So we wanted graduate students.

There had been a combined postdoc/predoc program that Victor McKusick had started many years before. When they split, we had actually three graduates of that program. One of them was Cheryl Corsaro,[57] the other was Lou Kunkel,[58] and the third was Nancy – who was married to Lou first but they divorced. I can't remember her name. That program was discontinued when the NIH decided that they were going to fund postdoc programs and not have combined programs anymore, so it was converted into a straight postdoctoral program. I must say that a lot of the people who were in the program – those are our three successes – but there may have been ten or twelve others that never got a degree. It was successful because of those three but not for the others.

I thought that we could do this because NIH has been very interested in training physicians to be scientists. So their physician awards – we try to get people who finish their residency training to get early K Awards[59] and things that will help them become scientists. The reason for that is that human biologists, which is what physicians are and what medical schools teach, are really in a terrific position to do good science. They're better than most people trained in traditional Ph.D. programs because there you get a very narrow, focused view of science. You don't get a big picture of an organism. So you may work with someone who's interested in eye problems, and you can learn all about the eye and know more about it than anyone else. But if someone talks about liver to you, you can't integrate.

The idea was that we would take people who don't want to be physicians and provide for them a human biology course of study so that they would get the background

that medical students get, but they can go on and use it for research. We were able to abbreviate the medical school program. It was very hard convincing the medical school that they would take our students into their regular medical student classes. They were very resistant at first, but we did it, and after they saw that these students weren't going to be disruptive and they might even contribute something to their classes, they became much more enthusiastic.

We had to shortcut, of course, the training, so they don't take physiology. They take histology with the lab, but they take pathophysiology. They're at a disadvantage without physiology, but they do it. They take pathology without the lab, but they take the lectures and they have a tutor. We've arranged that. The pathophysiology is the basic course of medicine, and it's so important. It's how everything functions. It's worked out very well.

The students sometimes think that it's too course-heavy because they're so anxious to be at the bench, but we just tell them that this is the time of their life – it's eighteen months – in which they are going to be pretty course-heavy, but they will find that that eighteen months is very helpful for the rest of their lives. And I think it is. We just heard the statistics. I think it's something like ninety percent. It's an enormous number of our graduates are in academic positions.

JC: This is a postdoc program?

BM: No. It's a graduate program. We also, as a tradeoff for biology – they don't do it anymore, unfortunately – for the biology department being involved, and they paid tuition for a year for these students and a stipend. [As a tradeoff,] they were teaching assistants in the courses in biology for a year, one in developmental biology and the other in molecular biology. It was very good. I sort of thought that since most Ph.D.'s are going to have to teach, they might as well have a teaching experience rather than this kind of medical school teaching experience. TA-ing here is not the same.

So that's the program. We got it funded and now what Hughes wants to do is have a program with pathophysiology. I mean, that's exactly what we've done, and it's taken twenty years for people to realize it. I thought it would catch on and we'd have a lot of competition, and it didn't. Because I guess it is course-intensive, and it was against the grain. It was very hard to get NIH to fund it because the reviewers had come through traditional Ph.D. programs, and they thought this was crazy. They couldn't see quite the value. But we managed, and we have managed.

One year there were only two – we had five years, and then we had only two grants that were submitted for reapplications. NIH decided that cycle not to fund any of them, and it was just awful. That's when I went to beg the dean at the time, [Richard] Ross,[60] to see if he could help us. He said he would certainly pay for all the students that were here, but that we couldn't take a new class. I said if we didn't take a new class, we would be dead, nobody would come any other time after that if they heard that there wasn't a class there that year.

What we did was to get an angel. I got an angel from the development office, and he supported three students, three excellent students. They've all done just very well. John Engelhardt[61] and Sue – I can't remember her name, but she just came here to give a seminar recently, and he's a real hot shot where he is. So we got these students, and the program went on, and the next year we renewed it and got it for the next five years, and so forth. So it's really great. I hope that there will be more programs like it now.

JC: You founded this program in human genetics in 1978?

BM: Mm-hmm.

JC: But it doesn't look like you had any students in your lab who were going through it.

BM: No, I didn't. I don't quite know exactly why. Dave Valle[62] manages to have students stay in his lab. But I was never able, as director of the program, to not support the preceptors. In order to get students in your lab, you sort of have to bad-mouth somebody else that they want to work with. I think it's very difficult. So that was part of it. I did have some rotation students. I lost one to John Gearhart,[63] and she's not in science anymore. I can't tell you exactly why, but I did have some rotation students, and they did work with me, but I didn't – maybe I'm too tough. I don't know. It's a combination of all kinds of things. I never fought very hard to keep them from going anywhere else because I thought other people's projects were really interesting. What I was doing was maybe interesting, but you have to feel like it is.

It's not an easy kind of thing. For instance, most people who work clinically -- and a lot of our postdocs come in that kind of way -- can understand working on cystic fibrosis,[64] and they can understand working on muscular dystrophy,[65] and they can understand all these disease-oriented research projects, but to say you're working on how X chromosomes are regulated in females in a clinical department is not –

On one hand, I've had some of the best students that anybody could have, and I'm very proud of them. On the other hand, I haven't had as many as I would have liked to have had. But then I never wanted to have a big lab, either. I figured two postdocs were just about what I could deal with. Do you know Carlo Croce?[66]

NC: No. How do you spell her name?

BM: Carlo, it's a he. He's at Jefferson [Medical College], I think. Anyway, he had a lab with forty postdocs, and I would say to him, "How do you know what's going on?" He says, "Barbara," – real Italian – he says, "There are only two good ones and I only pay attention to them." (chuckles) So, you know, it's – I would have liked to have had more students, and I would have liked to have more graduate students. I think that they would have had a good time.

JC: You did have two Ph.D. students – Cheryl Corsaro and Scott Gilbert.[67] How did you have them – what kind of Ph.D. program were they in?

BM: Cheryl was in the one I told you about, the combined postdoc/predoc thing that McKusick had. Do you know Cheryl?

NC: I do.

BM: Yeah, you know who I'm talking about. Scott came to me in a very interesting way, because he was at Homewood in the biology program. He was working with a young somatic cell geneticist who got himself in trouble politically with the head of the department.

NC: Who was that?

BM: Bob – I'm trying to think about -- he had worked with Frank Ruddle.[68] He was one of Frank Ruddle's postdocs, and he had come to do somatic cell genetics. Bob – and he got in trouble because of things like, he had a grant and they were taking money out of it as

common supplies, and he didn't think anybody should go into his grant. He didn't like the politics of the place, and they didn't like him for objecting. He was quite a smart guy, and he went on to Texas and did fairly well. He was very smart, but these things got in the way of what he could do to be really productive. He was sort of compulsive.

Scott started working with him, and when he left rather abruptly, he wanted to do somatic cell genetics and they allowed him to come to work with me, which was unusual because they don't usually let people off the campus to do that. But they felt responsible for him being – and he didn't want to go to Texas – and he couldn't because his wife was a medical student here, so he could not leave to go to Texas with Bob. That's how Scott came, and he was just a delight. He was absolutely great. He got a Master's in the history of science at the same time he got his Ph.D. here.

One of my students, Alan Beggs,[69] was a postdoc with me, so that was good. Spent a year with me. He's now at Harvard in Lou Kunkel's group, in muscular dystrophy.

So I did manage somehow or other to benefit from some of these students that were coming through. As I say, we wrote papers together, and so forth. But I'm not responsible for their Ph.D. degree.

JC: How have your relationships been with them over the years, while they were here and also after they graduated?

BM: Which ones?

JC: All of them, in general or –

BM: Well, the graduate students I usually see at the yearly meetings of the American Society [of Human Genetics] and they all slip back for cocktail parties, so we can see them. My postdoctoral fellows, some of them I have very close relationships with still. Dan Driscoll and Mimi, who I see every day. I'm still reading her papers. Dan Driscoll still sends me his papers. He's head of genetics in Gainesville at the University of Florida. Scott, Cheryl. There are a lot of them that are very good friends and have been for a long time. There are people who come but you don't want to – I can't say that everybody who has worked in my lab has been someone that I really need to continue a relationship with.

JC: You now have been at the same institution in the same department as your fellowship advisor, Barton Childs, for quite a long time. How has that played out?

BM: I'm not sure I understand what you mean. Can you be a little more specific?

JC: My understanding is that when people get Ph.D.'s in science, usually they leave the institution where they got their Ph.D.

BM: Yeah.

JC: And they see their advisor at meetings every once in a while.

BM: Well, Barton and I have been friends for years and years and years. I'd say up to recently I would go to see him for advice about things and to complain or – (chuckles). Sometimes when I'm happy, to tell him that. We've been good friends for a long time. He comes periodically, sits here and rants on with me. I've been giving him chapters of my book, even now, to read. He doesn't pay as much attention to details as he might have, but he has excellent things to say about the general organization and things still, so he's

certainly a resource for me.

Hopkins is a little bit inbred, I think, so a lot of people stay. I'm not unique in staying. I'm just unique in not quitting. (laughs) I mean in sticking to it. I think that one tends to stay at Hopkins as long as you can. There are people who leave because they have to leave because they don't have a source of support and they need to go. But Hopkins has been very clever in keeping you here. One of the things that has kept a lot of faculty are the tuition benefits for your children. I sent three children to school, Claude and I both, here, for not paying for it, the tuition. I mean, that's an enormous benefit, and you would hate to leave and run somewhere else.

My husband has always loved what he does and what he was doing. There were times when I thought about leaving and doing a weekend marriage and going somewhere else. I think that people here could probably thrive in other places because they'd be more unique. We have so many people who are stars in their own rights, so you may be underappreciated here; whereas you would be more appreciated elsewhere. Everybody who has ever left has told me that life is wonderful outside. But I think many of us [think], "Why move? Why leave?" But it's very hard to stay. In my day, I came back and then Claude was here. I didn't have a niche. I had to make something for myself. I didn't have a job; I just had an appointment. You have to bring in your funds and do that.

It reminds me of a story. Bob Cooke came back not so long ago, and we had a dinner for him, faculty who had been faculty when he was here. He was a tough guy, but a very good organizer. He brought a lot of good people, and a lot of good things happened when he was in charge of Pediatrics. Very creative time. I would keep going in to ask about things.

The first thing I asked him about, before I decided to do endocrinology, was whether I could go to NIH, because that's what residents did. When you finished, you went to NIH for training, like Dan Nathans[70] did and Don Brown[71] and such. He would say to me, "I have someone else in mind," kind of thing. Then I asked for a career award, to apply for one, and he said, "No, I have someone else in mind." He would always have someone else in mind. So I just did what I could do. One day he came to me and said, "I think you should apply for your own grant." Because I'd been on Barton's, that was comfortable. So I did. (laughs) That kind of thing. He did interfere every once in a while.

When he came back, I said to him – we all had a chance around the table to say something, and I said, "You told me every time I wanted to do something that I should just continue doing what I'm doing, that I'm doing it well, not to think about something else." I always wanted to feel more mainstream and was trying to. So I said, "I did what you said and I'm very happy. It's turned out just fine." (chuckles) You know what he said? In his typical manner, he says, "That's what I told everybody." (laughs) I said, "Thank you."

NC: He was a man with one piece of advice.

BM: That's what he did. Just keep doing what you're doing, you're doing fine. So, yeah, there might have been other things I would have liked to have done. I don't remember them very well at the moment, but I do remember even at the start that I would have liked to have rounded and made rounds with the house staff. They had so many men, actually, who wanted to do that that not everybody could. So you sort of got tossed aside through this process. It didn't make any difference, really.

JC: I'm wondering where Claude was in his career when you guys got married.

BM: I think he was an assistant professor when we got married, and he made six thousand dollars a year. I had made six thousand dollars as a fellow, so between us we

had twelve thousand dollars a year. (laughs) He had had a conversation with Cooke at the time about his salary, and he was objecting because his cohort, who had no more experience than him, was getting much more money. Cooke said, "That's because he's married." So Claude said to him, "Well, if I get married, will you pay me more?" And he said, "Yes." When we got married, he doubled his salary, so he made twelve thousand dollars, or something like that. With my little salary, we were doing well.

He was in charge of the lab in Endocrinology. We got married in '60 and by '65 [Lawson] Wilkins had died and Claude and Bob Blizzard[72] were the two that were co-directors of Endocrinology. So within four years he was a co-director. He had done research, and he was doing just research, so he had a clinical director. When the time came when Bob Blizzard decided to leave and they had to replace someone, Claude decided he might as well do the whole thing, and that's when he became in charge of the clinic. So that's where he was at the time. Always happy with what he was doing, always working on Saturday. He just loved it all. He came from France to be able to do this, so he always felt he had the right to do whatever he wanted to. He still enjoys it and doesn't want to quit. It's amazing.

NC: Maybe now's a good time to break if you wanted to. There's a seminar at noon?

BM: I thought that we had an IGM [Institute of Genetic Medicine] seminar today, but we don't. I had it on my calendar that Terry Hassel was coming, but I haven't seen him. So it doesn't make any difference. But we can.

NC: OK. In my experience, one tends to get kind of tired after a while. Maybe this would be a good point to stop for today and pick up tomorrow.

BM: Sure.

JC: May I ask a couple of specific questions?

NC: Oh, sure. Why don't you do that and then –

JC: The paper on penicillin resistance[73] that you published in the Hopkins magazine back at the very beginning of – when you were a resident. Was that your first scientific paper?

BM: Which paper?

JC: Penicillin resistance.

BM: No. I had done some work in Buffalo, actually, as a medical student that got published I think in the *Journal of Pediatrics* or something like that, that I did with the head of Pediatrics there. That was my very first paper.[74] I think the other one may have been my first one under the – I don't know what name I had. In my CV, what is the first Migeon paper?

JC: We could definitely find it. The other thing I was wondering, in the classes that you were taking at Homewood during your fellowship, were they populated by graduate students from the biology department, or –

BM: Yeah. Actually, one was an undergraduate course. I took McElroy's course in

biochemistry. I had had biochemistry in medical school, but this was an entirely different one because it didn't deal with humans. It was quite interesting, too. That was an undergraduate course, but the other two seminars were graduate seminars and I interacted with some interesting people. Yeah, they were all at Homewood. There wasn't anyone from here.

NC: The first Migeon paper on your CV is Migeon and Minchew, "Observations on Staphylococcus in the Pediatric Out-Patient Population," 1960.

BM: That's the first one, yeah.

JC: And who was on the founding board for the Ph.D. program in human genetics?

BM: Victor was, Barton was. I probably have that somewhere. John Littlefield.[75] I don't remember everyone at the time. It wasn't big. Maybe Wilma Bias. I don't remember who they were. Howard Dintzis.[76] I think Howard was, or he was certainly one of the first preceptors we had in the program. I don't know whether he was on the board. He was head of Biophysics here. He's still here. Renee Dintzis is his wife, and she teaches histology. He started up with the globins, I think, but he was in biophysics and interested in how molecules behave. He was head of Biophysics for a long time. Certainly, Phil Hartman was. He was, I think, the only person at Homewood for a while.

NC: Did you have more?

JC: I have more questions, but we knew we couldn't get to them all.

NC: All right. Why don't we stop here and we'll pick up tomorrow?

JC: OK.

Session 2 - June 3, 2005

V. Family Life; Barton Childs as a Mentor

JC: You had your first child, Jacques, in 1961, and Paul in 1963, and your daughter, Nicole, in 1965. Tell us about how you and Claude managed to raise your family and your scientific careers.

BM: We had help, and we helped one another, I think would be the answer to that. I was lucky enough to have a woman who stayed with us for thirty-five years and was the children's other mother. She didn't live with us, but she was able to move in when we needed to go away. But I had a husband that was very, very supportive of both of our careers and our having a family. I think that's terribly important, to find a spouse that is willing to do more than just verbally say they're going to help you, but will actually pitch in and do things. We had no he or she jobs, we just did whatever job was necessary, and we were able to get a lot done, I think, because of it. Does that answer it?

JC: I think so.

BM: I think it's terribly important to choose your spouse wisely because you do need help doing it all. And I think you can do it all. Helen Taussig used to come to our house on

occasion. She was a rather lonely woman, had no family. I remember a conversation with her. She would sit on the floor and play with my kids, play games with them. She said that when she chose to do science, she realized she couldn't have a family, but she was very pleased to see that we had evolved so far that women could do both. I think there's no reason that you can't. It's not easy, but if you have help and support, you can do it. Of course, I don't know what my children would say (laughs) or what they thought about the whole situation. My older son did marry a physician, so I guess it wasn't all that bad.

JC: It sounds like you're glad that you did both.

BM: Oh, yes. I think one of the most wonderful things one can do is to have a family, children. They give you challenges you wish sometimes you didn't have, but they enrich your lives. Of course, I adore my work, and it's nice to be able to do both. I honestly must tell you that I haven't felt it was an either/or kind of choice, because you can decide how much time you want to devote to whatever it is you want to do. There *is* plenty of time to do a lot of things in your lives, and if each has a priority for you, you can manage to do it. You may not do some other things that you might have done if you hadn't had a family as well, but I think you can work hard.

We went home so that we would have supper together. We always felt it was important to eat together in the evening. The children didn't like it that they ate at seven and not at six because they would have liked to have watched TV or done something like that, but they didn't get to do it.

We would bring work home, and then after they were in bed, we would continue to work, so you can find time to do things. I think a lot of people go home from their work and work at home, in our business. Some of them come back in, but I haven't noticed the lights on in labs and offices as much as it was when I first started in this business. I think a lot of people do go home at reasonable hours.

JC: How did you manage conferences and traveling?

BM: Well, if it was something that we were going to do together, then we would ask Ivy to stay with the children, and she would do that. If it was a conference that one of us had to go to, or the other, then we became the single parent and did whatever was necessary. My husband was absolutely marvelous. He would do everything with the children, from bathing them to anything that they required when it was his turn to be home with them. I of course would do the same when he was away. It wasn't a problem. We'd miss each other, but the children survived. It was nice having three all the same age because they sort of were company for one another.

Carpooling was something that I thought was interesting. We just couldn't do that. I think that parents do a lot of driving their children from one location to the other. I can remember my children as they grew up accepting an invitation for a birthday party and saying, "But I'm going to need a ride." They would arrange their own rides. They grew up quite independent that way. They didn't think that we would necessarily come home to do certain things.

And I found that there were so many parents who *were* at home with their children [and] were so nice to my children, too. One of the mothers taught my daughter how to sew. Another took my son to Gettysburg to a Confederate Army reenactment. All kinds of wonderful things that they would do, because they knew you could not. I don't know if that happens now, but it certainly was very helpful as we were raising our children.

JC: During the time you were having your kids, you were on Barton Childs' grant. What

expectations did he have of you?

BM: I don't know quite what he thought. He certainly never expressed any worry about my having children. I think I did enough work to not give him any cause for concern. There were people at NIH who were site visiting him at one point who did think that maybe I was having a few more children than was – they were worried a bit about my future plans.

JC: What was Barton Childs like as a mentor?

BM: Well, he's always been an excellent mentor. He's always there if you want to talk to him about something, and he has advice for you if you ask for it. I don't know what you're asking for in general. He was not a lab person, so I didn't learn techniques from him, but he's been terribly important in giving me an outlook about genetics, about what science should be like. I do remember his saying at one point that he felt it was awful that somebody would work quite hard and then try to become a professor and not get a promotion, and he felt that anyone who worked here and worked hard should be promoted and that we were perhaps making a small society in trying to decide who would not get in.

It certainly influenced my thinking about a lot of things, even about publishing papers. I have this kind of feeling that I'm not quite sure why the literature is being so protected from a lot of papers. They may not be perfect, but if they're not exactly the way someone thinks they should be, why not let the reader take a look at it and make his own decisions about the paper. You don't want anybody to embarrass you as a professor at Hopkins, but I'm not quite sure that I always understood – and he never quite understood – what all the criteria were.

So he's influenced the way I think about a lot of things. He's been a good friend for all these years.

JC: When did he set up his lab?

BM: He never really had a laboratory. He had space in the laboratory, and he often had a technician, but it was the fellows that ran the laboratory, or a visiting professor who came who needed some space and would be in the laboratory. So there was a laboratory, but Barton never worked in it. He was not a hands-on kind of person. He tells me that when he was in Boston as a fellow at one point that he was a real klutz, and they decided that he shouldn't be in the lab, or there was some talk about that. So he never felt quite comfortable doing that kind of thing. His forte was to analyze the data and the strategy for experiments. He could think about that very clearly. But he wasn't a hands-on kind of person.

JC: Where was his lab space?

BM: Well, when I first arrived, we were on the fifth floor of Harriet Lane Home, which doesn't exist any longer. I was there as a young faculty, too, so his lab must have been there. When he moved here, he did have a laboratory, I think on the tenth floor of the CMSC [Children's Medical and Surgical Center]. But before that, in the old Harriet Lane. I don't remember where it was. There was something, but I just can't remember.

VI. Migeon Laboratory; Cell Lines

JC: When did *you* first set up *your* lab?

BM: When I came here and was a fellow, I worked over in the Moore Clinic. Then I came back, and my lab initially was on the old Harriet Lane fifth floor. I shared lab space with office space that Bill Nyhan[77] had. I remember looking in the microscope and listening to Bill Nyhan dictate his sometimes personal letters to his secretary and wondering what I was doing in the same room as he. Then he left and I had the room to myself. He left to go to Florida, I think, as head of the department there. And I worked on the fifth floor of Harriet Lane until we moved over to the Children's Medical Center building. I had a lab there on the eleventh floor.

JC: When did you move into the Children's building?

BM: When it opened, and I don't remember the exact date. I should, because I have a drawing of the move, and they were pushing the patients down the hall.

JC: Was it after Nicole's birth?

BM: I think so.

JC: So after 1965.

BM: I think it was – maybe that was the year. Yeah, it's about '65 or so. That building still exists, but it's considered old and it's not really that old. They're building a new Children's Hospital now.

JC: We were wondering if you could walk us through your lab space on a typical Monday morning. Give us a tour.

BM: Well, I was on the eleventh floor for maybe ten years, and then I moved down to the tenth floor into much nicer space. I had a laboratory that – on one hand, it was the laboratory that I used for molecular biology, biochemistry, and some cytogenetics. Then on the other side of the hall was the laboratory used for cell culture, and the kitchen that was associated with it. I had a darkroom with a microscope off the molecular biology lab.

On a Monday morning? Oh, Monday was a big day. All the cells needed to be fed. I usually had a technician that took care of the cells and did experiments with me for that. Then I had another one who was sort of a support for the molecular lab, sometimes helping fellows if they needed help, and often doing other experiments. I sort of carried out some independent work at the same time my fellows were carrying out their work. So we would be feeding cells. It was always a buzz, on Mondays anyway.

I had only a couple of technicians, actually, in the cell culture room throughout my life. I've had very long-time employees. The first was a young woman who went with George Thomas when he started his cytogenetics lab. The other was Joyce Axelman,[78] who worked with me until a few years ago, and then she went to work with John Gearhart. She does the stem cell work with him now.

Joyce was very much of an expert in cell culture. We worked out original techniques together. After that, she would set the stage for me, so if we had experiments to do and things that we'd planned, she would set it all up and then call me to do the fun part of it with her. I always sort of kept my hands in. I made hybrids, I cloned cells. She could have done it without me, but I didn't want her to. I think she does all the stem cell stuff for John downstairs, except for the stuff that his fellows do. He's not a hands-on person, so she can do that. It made it a lot of fun for me. She would freeze the cells, and she would do all of the ordering and making sure that everything was there, and I just had

to come and do the experiments.

NC: What was involved in the feeding of cells?

BM: Feeding cells is just changing the medium. They're sitting in a Petri dish. We had many different kinds of medium because each kind of cell had its own requirements, and you didn't want to mix things up, either. They would sit in these round circular Petri dishes, and all you would do was to aspirate off the old media and put on the new medium. She would make up a ton of different media, and the fellows were always supposed to tell her what they would need for their cells. Because I always had them take care of their own cells.

One time I had one postdoctoral fellow complain that Joyce had contaminated his cells. I said, "I'm sure she hasn't, but to make sure she doesn't, I think you should make your own medium, the final sterilization of it." Joyce would make up the medium, but the final sterilization was always done by the people who were taking care for their own cells so that they wouldn't have a chance to blame anybody else for that.

We had different media for hybrids and different media for ES [embryonic stem] cells. Everything requires different kinds – that's really tricky in making sure – we have the recipe book, and everything was written down. She would take and put it on a sticky [note] and put it up over the hood so that everything would be added in a meticulous fashion.

We took pride in the fact that our cell culture lab had very little problems through the years. We didn't have contamination. That's not only bacterial contamination, but a lot of people contaminate one cell culture with another. That's easy to do. So we were pretty compulsive about how we changed the media. We waited between cell lines, and we didn't put them on the same shelf in incubators. Everything was pretty – I think you have to be very compulsive to do cell culture. I do think so. So it would work well.

Then, of course, we had the people who would take our cell cultures, having ordered them a few weeks before at times so that we could grow up enough in order to assay them. They would take and harvest them, meaning sometimes trypsinizing[79] them to get them off the plate and into a cell suspension that they could then work with. Other times scraping the things off the dish if you didn't need to retain the form of them for the assay that you wanted to do. They were using the cells as a source of protein or DNA or RNA, I mean through the years, so each of them had a different protocol, of course, for getting it. So there was a lot of activity.

We had a lab meeting – I think I introduced lab meetings to our department, in Pediatrics anyway. They were held in most other departments everywhere, but it was when I went on sabbatical in '76 in Don Brown's lab that I saw how a lab should report to one another. He did have these marvelous lab meetings, so I started them [at Hopkins]. Shortly after I started them, everyone else started to have lab meetings. So we would have them on Thursdays, and they could last for hours at times. We had a great time, because everybody would sort of report on what they had done, show you pictures. It's a lot of show and tell. Then we would plan the strategy for what should be done next.

JC: In terms of people, your lab generally had yourself and two technicians?

BM: Two technicians, two postdocs, and often students who would wander in. When I first started in this business, it was very exciting for medical students to come and do one of their rotations in a research lab, so you often had these wonderful medical students coming to do research with you because they wanted to see what it was like, and they were very excited by it. I find that, unless you're in the MD-PhD program now, very few medical students think about doing that kind of rotation.

It used to be that college students wanted very much to spend some time working in the lab during their summers, so you would have often a lot of summer students who wanted to come. Now I think medical school admission forms require you to be tutors or to volunteer, and they don't put as much emphasis on having had any kind of research experience. So there's been less of those kind of students. If they come now, they usually come from other countries. They write and say I would like to spend the summer with you.

I loved summer research students because we'd often get an enormous amount accomplished in the summer. They might even have a paper at the end of it, which would surprise them. It wasn't all their work, but they were part of it. We could go on with it, finish up some ends and they would feel very good about having contributed to something. So I always had some students around as well.

JC: In terms of projects, did you assign projects to your postdocs, or did they come with their own?

BM: I have never, I don't think, had a postdoc who came to me with an idea about what they wanted to do. I may have had one or two who wanted to do something entirely different from what we were doing, and I just suggested they might have to go somewhere else because I couldn't support that kind of effort. You have research funds that are focused, and you have to be rather focused.

Most often, we would talk about what project they could work on. Some of them were encouraged to write grant applications before they came about that project so that they would have to think about it and try to get some kind of plan in mind. I think most often they didn't know enough about the problem in order to devise the experiments that they wanted to do. But there was no question.

Scott Gilbert was very interesting. He was a graduate student of mine, and I would say that I didn't give him an idea of what to work on, he was one who came with a thought. I really liked the idea because we were looking for a way – and we may have discussed this – of getting epithelial cells to grow. At that time it was before growth factors and very difficult to grow anything but fibroblast. You'd take tissue from any organ and you wouldn't get the tissue that was characteristic of the organ, but you'd get the interstitial tissue to grow up.

He came up completely on his own with this wonderful idea that he could inhibit the growth of these fibroblasts and let the differentiated type cells grow up. The way he did it was to – he knew about an enzyme – I didn't know what it did (chuckles), D-amino acid oxidase, which allows you to use the D form of amino acids. The L form is the common one, but there is an optical isomer that is D.

It would allow you to use a D form, and he knew that fibroblasts didn't have that enzyme, so he suggested that what one could do was to deprive the media of – at least one, and he chose valine L-amino acid and put a D-amino acid in there instead, so it was D-valine that was in the media. Then you had a dialyze serum to get rid of any source of L-valine. Then to feed the cells with that, use that as the nutrient medium.

What happened, he was absolutely right, the fibroblasts couldn't grow, and we could start seeing lung cells and kidney cells, which were the tissues that we were using for these experiments. It was quite exciting. He had a cover paper[80] in *Cell* describing this work, showing how he could select for [epithelial cells]. I think Jim Watson[81] used the picture, the cover, for the first book that he was involved in, in the series of books.

NC: *Molecular Biology of the Gene*?

BM: Yeah.

NC: Not the '65 version.

BM: Well, I don't know if it was the first edition. It may have been a later edition, but it was in that for a short time [possibly the 3rd edition, in 1976].

NC: When did Scott get his degree?

BM: I don't know. It's in my CV.

JC: When did he get the cover on *Cell?*

BM: I have it somewhere [May 1975]. I don't have it on the wall, but he did. Scott was very clever. He won the *Nature* writing contest, he did all kinds of things. He was very, very clever. It was probably in the seventies, early seventies, sometime like that.

Anyway, it worked. The only problem was that the cells that we selected didn't go on for very long. They needed growth factors, and we didn't think about doing that. But it was used for quite a long time. GIBCo [Grand Island Biological Company] actually made up a medium that they called D-val.

NC: GIBCo?

BM: That's where we get our nutrient medium. Grand Island Biological Company, GIBCo.[82] I don't know whether they're still called GIBCo or not, but that's who sold us most of our media, and they did have special media.

JC: Where did the cells originally come from?

BM: Oh, where did the cells that we used come from? That is an interesting question. I, for many, many years, have studied human embryos. I have, even now, cell lines that are derived from many, many years ago from human embryos. We used to get them at the time of surgically induced abortions, terminations, or spontaneous terminations. I had collaborators in the departments of OB-Gyn who would help me get those.

We had nothing ever to do with the patients. I didn't even know the names of patients, and I had no way of getting back to them even if I found – I once found something interesting that I really wanted to tell the woman about, because it was chromosomally abnormal, but you can't do that kind of thing. There was always an IRB, an Institutional Review Board, and we did have permission.

For a while we had to ask the women ahead of time, before they underwent this procedure, for permission. I did a study at the time. It was quite interesting to see what their reactions were, and it was so disturbing to them that I went before the – because they hadn't thought about this. They thought they were having a termination, and they didn't think about it as a real – anything that would have value. When you go and request the specimen, it was disturbing to some of them.

We brought this before the Review Board and told them that these were really discarded products of conception, and we really shouldn't have to ask permission to use them because they were only going to be disposed of, put into an incinerator. So we arranged with Pathology that we would get it as soon as they had a chance to verify the fact that there was fetal tissue that had been obtained from the procedure, and then we would take it. It was often not intact in any kind of way, but we could identify tissues.

We used those tissues for so many studies of early development. It's been very,

very useful. We obtained all kinds of chromosome abnormalities that wouldn't have survived to being born that one could look at, and they were a source for me for triple-X's and triploid organisms with sixty-nine chromosomes, that I used for many studies. So we always had sources of fetal tissue, and that's what we used for these experiments. It's hard enough to get adult surgical tissue.

NC: Did you ever experience any objections from any member of the public to your work? I'm thinking about parallels with the stem cells.

BM: No. I'm writing now about some experiments, and I just say we have IRB approval. I always say we aren't a part of the decision to undergo this procedure. I haven't had editors or anyone question it. My funding has been from NIH, and they didn't question it. I'm not sure what will happen in the future, but up to this time – people tell me it's very, very hard to get these specimens and I have some, and I'm trying to figure out a way of getting them to places where they might be used again.

The wonderful thing about freezing cells, I can tell you that I had – I studied the first Lesch-Nyhan[83] syndrome child. I was on the house staff at the time, so that was very, very early. [William] Nyhan[84] was here and [Michael] Lesch[85] was a medical student, and they had this child on the ward. They asked me if I would look at its chromosomes to make sure it didn't have a chromosome abnormality. We just need to look at chromosomes, and you got to look at all kinds of things. I did, and it was a normal male; but then they discovered that it had this interesting defect in purine metabolism and didn't have the enzyme HGPRT. The first child was one of two brothers. One boy had been killed in a car accident, and the other one was on the ward and was a baby who was urinating these crystals, these uric acid crystals, and that's how he came to their attention.

I think it was about two years ago that – I studied this family later on, and it was a wonderful – It's an X-linked enzyme and we showed that there were two populations of clones. There was a variant. In this case the variant was the absence of the enzyme, so we could show that there were clones. Like G6PD A and B before, but we could show that the heterozygote had clones with the enzyme and clones without the enzyme and that she was actually a mosaic. I really did study the family and got to know them, and the grandmother.

We did a big study on cell selection and whether cells that had the enzyme had an advantage over the cells that didn't have the enzyme, which is true in blood cells, not so true in fibroblasts. That kind of thing. So I did study this. The early *Science* paper on the heterozygote has two clonal populations, with HGPRT.[86]

Bill Nyhan, about two years ago, asked me if I had any cells from this family because he wanted to know what mutation they had, so I took the cells out of my refrigerator. It's like thirty-five years later. And out popped the mother and her son, and they grow as if they – so I sent them to him and I'm sure – he hasn't gotten back to me to tell me what the mutation is, but I know he knows. (chuckles) It's very exciting to think that you could keep things stored many years after the son is no longer living. We have all these specimens on people who never survived, yet they're so useful because they divide beautifully.

We never kept cells in the incubator because human cells have a limited lifespan. They live something like fifty generations. Leonard Hayflick showed that many years ago [the Hayflick limit].[87] If you keep them and keep subculturing them and transferring them, then you wouldn't have them. But whenever we got a tissue, we would culture it in many dishes, and then we would take each of these originals and freeze it away very early, in the original Petri or in the first subculture. We would reflood the Petri and let things grow up and refreeze again. I have stocks of twenty vials of a tissue very early before it's been

subcultured very much, so that's why I can bring it back and it can be living, because it hasn't used much of its lifespan and still has the potential to go on. A good secret is not to keep things in your incubator, keep them in your freezer. They last much longer that way.

Whenever we finished studying a thing we would refreeze it and put it back, so we were always replenishing. I have this freezer full of thousands of cells that I'm trying to find homes for.

JC: I'm wondering when that IRB decision was for you not to have to ask the women before their abortions.

BM: It was, I would say, around '76, '77, because that's what I had Karen [Jelalian] doing. She was interviewing these women while I was at the Carnegie [Department of Embryology at Hopkins], and that's about the time, about '76, '77. It's been a long time. When the board would question that again – Tom Hendrix[88] was in charge of the board for many years. He had sent me a letter, and I would just send the letter back, in which he had made the decision that it was not needed, that they were just discarded. Do you think it's not a good idea? Do you think it would be better to ask someone?

JC: I've never been anywhere near any of that. I have no idea.

BM: Well, you know, it's quite interesting, because the permission form that I do sign for specimens says that when I do get permission, that we're going to study this genetic defect and that we will dispose of the cells when we finish. Now, *I'm* finished, but are my cells finished? Well, the IRB decided that we could grandfather cells if you have original permission, and so that I should now be able – they're legal for me to give to someone else, even though I'm not sure how people would feel about knowing that their cells are still being studied.

From the people that gave me permission, I would say they would be very happy to know that we were using them. I don't think they would like it if we were using them to make a clone of somebody, but the fact that we're using them to study their disease, or [that] their disease is useful to enlighten us about some general regulatory mechanism, I think they would be very pleased.

I don't think that these women, in retrospect, would be unhappy. I just think at the moment when they – it was a very hard decision for some of them to make, to give up a fetus. But we were making it more problematical for them when they made the decision. I think that if asked two weeks later, they would have said, oh, fine, go ahead. Do you know what I mean? I may be wrong. There were some who it didn't bother at all, but others we could just tell by the way they hesitated, looked at us. And some said no, which is – that's fine, too. If they say no, it's no.

I've always felt, and I do feel that material that isn't going to be used shouldn't be thrown away and should be put to some kind of good use. These were somatic cells, they weren't germ cells. They were skin cells, organs. But you don't really want to think about it, I think, if you're in the process of – yeah.

JC: We were wondering if you hold an opinion on abortion in general.

BM: Yes, I do. (chuckles) I don't think it's much different from at least fifty percent of the people. I think it's a woman's decision to make, and I resent other people thinking they have a right to get involved in it.

I would always tell technicians that I interviewed that I was working on aborted material, and I had a couple who said they couldn't do that. So we didn't hire them.

Nobody who came into my laboratory didn't know that there would be cells from aborted fetuses there. Some of them said they wouldn't want to have to handle the tissue themselves, but they would not mind using it, analyzing it, once they were cells. They didn't want to handle the dissection, which is what we did. I mean, we were dealing with real tissue. It's not necessarily a pleasant thing to do, but a lot of people do this with mice, and you can have the same kind of objectivity. These little mice are sort of cute, too. (chuckles)

VII. X Inactivation; Housekeeping Genes

JC: I was wondering when you started to use – you've used several different animals, mice and marsupials. When did you start using non-human tissue as well?

BM: When the questions required us to use them. The first time was to use mouse cells because I needed to separate one X from the other X. We needed to have cells that had an active X versus the inactive X, and the way to do that was to make hybrids so that you could devise ways of keeping either the active X in the hybrid or the inactive X. That's the way you'd know what genes weren't being expressed on the inactive X. You might treat it with things and then ask whether you could re-express something. But you needed not to have the other chromosome in the way. There weren't as many polymorphic markers, and now you might devise experiments in a different way to ask those questions.

In the March 17th issue of *Nature*, they talk about genes that escape X inactivation that were done with nine of these hybrids, with an inactive X, so it's still being used for the same purpose.[89] We started with A9 mouse cells, and I can't tell you how excited I felt because I knew from John Littlefield, who was in Boston at the time I started to do this. He came here as our department head subsequently. He had isolated this A9 cell line, and it was a cell line that lacked an enzyme that was called inosinic phosphorylation something or other.[90] I don't know what the name was.

Of course, I had been working with Lesch-Nyhan cells, which were HGPRT deficient, and when I realized that the two cells had the same defect, the mouse had – it's an HPRT mutation, actually [the enzyme is HGPRT; the gene that expresses it is the HPRT gene]. It was a lot of fun to do, to know this. I made this discovery. If I had been a better biochemist, I would have probably known that. I mean if I could have visualized where these things were in the pathway. I didn't at the time. I just knew you could use HAT medium,[91] which is a special medium that will select the cells that have the enzyme, and you could use isoguanine or these analogs, 6-thioguanine, that would kill cells that had the enzyme so you could reveal the mutants. John Littlefield had shown that that was possible, and Szybalski before him.

NC: Waclaw Szybalski.[92]

BM: Yeah. So it was very useful. Then we just used it on our Lesch-Nyhan heterozygotes to see – because they select against this mutant allele, the heterozygotes do in their blood, so they end up during the first decade of life without any of those cells anymore, which was always a problem for the clinician because he would try to see who was a carrier of this mutation. They would look for the enzyme in the blood, and they would get the same levels of enzyme in the blood of the carriers as they did in normals.

What happened was, using my G6PD markers in this wonderful family that was segregating two mutations. One was just the AB variants, and the other was HPRT. They could show by making clones and selecting them on the basis of which G6PD variant they expressed, whether it was A or B, looking at what the HPRT marker was. What they found

is that, although in their skin they were AB, in their blood they were only B, because they had completely eliminated the A population. That's why they looked like they had normal –

That's why a lot of women are mosaic, because the really are in some tissues. In others they've eliminated cells. The skin they don't eliminate because they can share. The little enzyme that's present in one cell gets transferred through gap junctions[93] to the cells that don't have the enzyme. Therefore, you don't have a deficient cell, and therefore you don't select and eliminate. So if you wanted to know who was a heterozygote, you had to look at skin and not blood cells. We are fascinating the way our cell populations interact with each other.

That's when I started working with mice, to get back to your question. We discovered that, and it was very exciting. Stan Wolf[94] was in my lab, a marvelous postdoctoral fellow who came from St. Louis, from Wash. U [Washington University], from David Schlessinger's lab,[95] actually, and had worked in RNA metabolism there. He came to work on X chromosomes. It was just after I'd taken a sabbatical in Don Brown's lab, where I started to learn about how to handle DNA and RNA and whatever. I was trying to isolate the Barr body by making it heavy with BrdU and seeing if I could get it out as a peak on cesium chloride.

JC: What's BrdU?

BM: Bromodioxyuridine. It's an analog of thymidine. It's heavier. It would produce a satellite in cesium chloride, which is a way to separate out DNAs. It didn't work, but I spent the year trying to do it. [laughs] Then Stan came along and we decided we were going to use brute force in order to try to get – we wanted to get some inactive X DNA in our hands so that we could look at it. That's what we wanted to do. I was going to do it by isolating a Barr body, but we did it essentially – and Stan was the one to do it – by taking a 4X cell line and an XY cell line and making libraries, and then trying to see if we could get X-linked genes on the basis of showing that whatever clones that were in the library hybridized better to the 4X than they did to the XY cell line. We started with 4X's to enrich our library for X chromosome sequences, anyway.

He was able to get out some single copy clones, which gave us our full look at the X, and that was a *Cell* paper on cloning, on cloning the X,[96] which really got reported in a paper from Hong Kong somebody brought back for me, as if I was cloning individuals. (laughs) We were only getting cloned sequences from the X chromosome, but it hadn't been done, so we got the first cloned sequences. They were not genes, they were pieces of DNA. But we were able to look, and we could tell that we didn't have methylation differences in every gene on the X chromosome, which was one of the hypotheses that was around.

It wasn't until we looked at HPRT as the gene was cloned by a group in California with whom we collaborated, to look to see what that looked like on both active and inactive X. There, there was a difference in methylation, and Stan was very quick to realize that it was in the CpG island,[97] which is in the promoter region of the gene.

It's interesting, because somebody else was doing the same experiment at the same time. They saw the same differences between inactive and active X, but they didn't appreciate where these differences resided. Stan really understood that these were – we called them CpG clusters, and we reported those as control regions for housekeeping genes and showed the difference – the X inactivation has been such a marvelous model to look at the significance of certain things. It's where imprinting first came out of and where DNA methylation, the importance of promoters and CpG islands came out of. Because you have a chance to look at two chromosomes in the same cell. One is active and one is not.

It's just such a unique situation that we've learned so much. We learned through the hybrids we could isolate the chromosomes, that when we reactivated the methylation on the inactive X at the HPRT locus that we could reactivate the gene, and we knew, in many clones, what got reactivated, what got demethylated, in order to reactivate the gene. There were differences on both chromosomes throughout the gene, but it was the differences in the CpG island and the promoter region that was important for turning the gene back on. It was really very enabling, and one thing would sort of lead to the other.

Stan did some nuclease hypersensitivity assays[98] and showed that there were hypersensitive sites when it was on the active X, and they were not hypersensitive when they were methylated on the inactive X. He could do that for several genes along the chromosome. That's one of my favorite papers in *Nature,* on the CpG clusters.[99]

At the same time, Adrian Bird[100] called [them] HPa islands. He didn't study X inactivation, but he had cut DNA with a methylation-sensitive enzyme and showed that there were very tiny fragments, tiny HPa2 fragments, which is why – he called them HPa2 islands [HPa2 codes for histone acetyltransferase, a particular yeast protein]. We sort of shared what they ended up getting called. They got called islands from him and CpG from us. (chuckles) Which is what they were. But I still think CpG clusters are better than islands because they are clustered CpG sites. They're about 1KB on average, and they're found in the promoter regions of the first introns of housekeeping genes or growth factors. They're found elsewhere on the chromosome, too. There are several of them within factor H, which is a very big gene on the chromosome.

It was the first look that we had at genes that were not tissue-specific. Everybody thought, since globin came out first, it was the first gene that was cloned and looked at, that everything was going to be a globin gene, with a TATA box and a CAT box[101] and room for transcription factors. Well, housekeeping genes were genes that were just so different. People didn't believe them at first when you said there was no TATA box and no CAT box there. It had a CpG island in its promoter. They're genes that are constitutively expressed, they don't have to be turned on.

They're called housekeeping genes because they are expressed from – they're unmethylated in sperm and they would be able to be expressed if it wasn't condensed to chromatin. As soon as they get into a cell, they get expressed. They're not meant to be turned on and turned off. They can be regulated in many ways, through degradation. One of the most marvelous things – and I'm not still sure why it's true – but HPRT activity is two hundredfold [greater] in cerebellum than it is in any other tissue.

Well, and that's where Lesch-Nyhan individuals have problems, because it's really a salvage enzyme and you could do without it in many places, except the heterozygotes tell us as we're eliminating it from their cells that it must have some function as an energy source or something because it's not competing very well with the normal gene when it's mutated. But nobody would have thought that this gene would have been important until you got Lesch-Nyhan syndrome.

Their problems are cerebellar. They have cerebral palsy.[102] It's a fascinating syndrome. They may or may not be mentally retarded. They have bright eyes, if that's the kind of mental retardation they have. They're so uncomfortable all the time because they have this pileup of uric acid, and they have cerebral palsy, they can't sit very comfortably, they have gout,[103] they have this fascinating need to mutilate themselves, so they tear at their lips. It's quite interesting that a single gene can do all these kinds of things.

And it's fascinating to think that their mothers show nothing, absolutely nothing of this, except a very few females who skew X inactivation for one reason or another and they end up with too many cells expressing the mutation. They get sick _____ [interference - inaudible] Maternal heterozygotes are very protected. They eliminate the

bad cells from their blood, and they share the goodies in their skin, so they never get into any kind of metabolic problem, whereas their sons are very sick.

JC: How often does this disease occur?

BM: Oh, it's a rare disease. I don't know what the frequency is. One in a hundred thousand, or something like that. You know, when you're interested in it, you get to see a lot of them. I think I've studied about fourteen families, and one is a very extensive family from Texas that had both the G6PD variants and the HPRT variants. It was one of these sort of pathetic situations where we studied one branch of the family and thought we knew everybody. We told the heterozygotes they were heterozygous so that they would know they needed amniocentesis. But they didn't tell us about another branch of the family, nor did they communicate this to another branch of the family, so we got another couple of affected individuals that could have been prevented. People don't want to talk about their problems too often.

JC: So you've done some genetic counseling.

BM: Oh, I always felt responsible for anybody I studied. I would do prenatal diagnosis, too, in our lab, with no cost to the patient if it was someone that we had studied.

JC: Just to go back. The paper that you're referring to that you told us was your favorite paper, was it your 1985 *Nature* paper with Stanley Wolf titled "Clusters of CpG dinucleotides implicated by nuclease hypersensitivity as control elements of housekeeping genes"?[104]

BM: That's the one, yes.

JC: Where is Stanley Wolf now?

BM: Stan left here to go to what was then the Genetics Institute in Boston. It's now owned by one of the drug companies. He worked [there] for a good length of time. He cloned IL-12, I think, or IL-2. I always get it sort of confused, but one of the important interleukins.[105] It was in testing for cancer, as a therapy for cancer. He's still there, but it's changed position, and he's changed projects because the company decided that it didn't have an effect by itself in cancer and they didn't want to go along with trying to let him spend time finding the cofactors that might work with it, kind of thing. That's what industry is, and if one company discovers something, you will probably not end up working on that project any longer and do something else. So he was looking at the effect of IL-2 or something in hepatitis, a little upset that he couldn't go on with the cancer trials.

He's done very well. He's on the staff, and he has a big lab that he controls there. He has a son who was born with a heart defect and is taken care of at Boston Children's Hospital, which weds him to Boston.

JC: How did you determine that methylation locks in the inactivation but doesn't initiate it?

BM: Well, we're going on with your question of how we got involved also in marsupial cells. First of all, the experiments that – I think the marsupial cells are probably the best example of that. We knew that X inactivation was leaky in marsupials. They have a different kind of inactivation. It's the same basic mechanism, but the father's X is always

inactive in every cell of the fetus as well as the membrane.

It was interesting to know why. We knew it had X inactivation. In the tissues themselves you could see that you had one active X and which one it was, but at some loci you'd see a little bit of expression of the other one so that everybody knew that it was a little leaky. What we could do was to find some animals that had variants so that you could distinguish one G6PD from the other, and that's the way they knew about the leakiness, because they did have that kind of polymorphism and you could see that the father's X was the one that was always turned off.

David Kaslow[106] came along, and he was a super guy, too. He took these cells that we got a hold of and he cloned the G6PD gene, using the information we had about the human one, in the marsupial. It was the first marsupial gene cloned. Up to now, there hasn't been an awful lot more activity on that front. He also got part of the HPRT gene to look at, too.

JC: Kangaroos, wallabies, opossum?

BM: No, no, no. Opossum. We didn't go to Australia. The North American marsupial. You know, it's really road kill, the opossum. There is a place where we were able to get little ones as well, and a family, and all kinds of things. There's a Southwest Foundation [for Biomedical Research in San Antonio, Texas] that was breeding a special kind of opossum as well. So we had sources of tissue to use. We just cultured them like you do anything else and grew them up. He did use the tissues and such to clone the gene, and then when he did, they had a CpG island, too, but he studied it and found that it was unmethylated on both chromosomes.

We knew also that in the tissue it was inactive, so if it's inactive and yet when you look at methylation, you have no methylation in the CpG island, and this is leaky; it tells you that methylation is responsible for keeping it from being leaky, but it's not responsible for the inactivation because you have that anyway.

We took those cells from the marsupial, and they were so different from human cells, because when you put human cells in culture, or even in the hybrid, an X chromosome that's inactive is going to stay inactive. You can culture it all you want as long as you don't add a demethylating agent or something of that sort, and it's going to stay perfectly stable. But we took these marsupial cells that, in the tissue, were inactive. I mean, one chromosome was active and one not. Put them into culture, and within two subcultures, we had both genes being expressed equally.

NC: You have to actively maintain the inactivation.

BM: You have to, yes. If it isn't there, it will leak. Now, not the whole chromosome. If you use a demethylating agent, it doesn't get the whole chromosome, it gets sporadically enough. I don't know how many you need. You need to get quite a few. You don't have to get all the CpGs within the island unmethylated, but you need to get enough to get some expression. The more you remove, the better it is.

I think you sort of hit the chromatin region around the chromosome with these demethylating agents, because we found that when we demethylated G6PD, we got two other genes that were close to them in the pouch, but we wouldn't necessarily get PGK [phosphoglycerate kinase] or HPRT because it would depend on how long you treated them.

Demethylation is very sporadic in its maintenance. But in the case of the marsupial, the fact that we could turn on these genes, HPRT and G6PD, without doing anything, just by culturing them, tells us that there must be some tissue-specific factor that's keeping it

inactive in the tissue, and as soon as we put it into culture and start it dividing that we're losing that particular function.

There's no question about this, now that people are looking at ES cells and following, you can induce X inactivation in the ES cell by –

JC: ES being?

BM: Embryonic stem cell, mouse embryonic stem cell. You can induce it. You can take an undifferentiated one and you differentiate it, either by adding retinoic acid or – It's maintained undifferentiated on feeder cells. If you remove the feeder cells, then it starts differentiating, and within four days, you get X inactivation to occur. So now people have been following these events that occur, and it shows that DNA methylation is a late event, that it comes after the chromatin is inactive. It really is a maintenance factor and not the initiator. I'm sure later I will think of a lot of other reasons I know that it's not responsible for initiating it.

VIII. Mapping Genes; Technological Change; TSIX; Mosaicism

JC: Let me go back on my list rather than forward. When did you first map genes on a chromosome?

BM: Oh, as soon as you could. (chuckles)

JC: When was that?

BM: We were in the hybrid business very early. The nice part of where my lab was in the CMSC is that I was very close to the clinical services, and of course my friends in cytogenetics would tell me when interesting patients came in. We had lots of X–autosome translocations, and they were a wonderful way of mapping genes to regions of the chromosome because you could follow a chromosome.

We started very, very early, as soon as [Mary] Weiss and [Howard] Green had shown that you lose human chromosomes in the hybrid, and we had made hybrids.[107] They came up with the idea that there was a chromosome that – I'm trying to think. My little Lesch-Nyhan, [patient name], was the – I shouldn't say that because we're not supposed to talk about patients anymore, but he was the original Lesch-Nyhan. We put him with a cell line that was called clone 1D and it lacked thymidine kinase. This was just right after the Weiss and Green experiment. I had these things in my incubator, and I had Lesch-Nyhan cells. Mary Weiss was at the Carnegie, and she gave me her clone 1D cells on a little cover slip, and we took –

NC: The Carnegie Department of Embryology, here at Homewood?

BM: Yeah. She was doing a sabbatical there for a year after she finished her work with Howard Green.[108] We put it together and noticed that I ended up with hybrids that had only a single human chromosome in it so you could try to map it. It was marvelous because the chromosome that it kept was chromosome 17. We didn't know at the time because we couldn't identify it by banding. But we could tell on its morphology that it looked like a chromosome from what was called the E-group at the time.

We thought that since the cell line that I used lacked thymidine kinase, and we were keeping just a single human chromosome using HAT medium, which is the medium that not only selects for Lesch-Nyhan enzyme-positive cells, but also for thymidine

kinase-positive cells, that it could be thymidine kinase. So we used BrdU [bromodioxyuridine] to select against it and watched it disappear, and we could bring it back. So we concluded, and we reported that actually in *Science*. I don't know what year that was.

JC: Was it the seventies?

BM: Seventies, yes. Early. Later on we went back as soon as you could band chromosomes and O[rlando] J. Miller[109] was able to identify it as 17.[110] It was the first autosomal assignment to a chromosome, chromosome 17, which has been obscured because Victor McKusick, meanwhile, a month before our paper, published a paper in which he identified chromosome 1 as having the Duffy blood group on it, based on an uncoiled region of the He always went around saying that his was the first autosomal assignment, but we really came a month later with the first hybrid assignment, using hybrids to identify the specific chromosome. Now Mary Weiss had made it all possible by showing that you could lose human chromosomes, and it set up the likelihood that you could follow genes along the chromosome as they got lost and be able to map them.

Am I clear? When you hybridize a mouse cell with a human cell, they're on different spindles essentially, but there's only one spindle that gets there in the hybrid that's able to proliferate, and that's the mouse spindle. It sets the cell cycle. So human chromosomes have a hard time getting on that spindle in time to get into the daughter cells. So you lose human chromosomes. It's characteristic.

JC: I don't think I know what you mean by spindle.

BM: Oh, that's mitosis.[111] It's the spindle where the chromosomes –

JC: In the middle?

BM: In the middle, yeah. The metaphase plate is right there in the middle of the spindle. The spindle holds the chromosomes at their centromeres, so it guides the –

JC: So it's the spindle fibers that come off the ends of the chromosomes.

BM: Yeah.

JC: I guess I've always heard it as spindle fibers.

BM: Well, maybe that's the proper way. I don't know.

JC: I would guess you have the proper way.

BM: (chuckles) I don't know. It's just that they get left behind, so you end up keeping only the chromosomes that contribute something vital to the cell. If you start out with a mutant cell, mouse cell, that lacks thymidine kinase, and you put it in a medium where you need to have thymidine kinase, it's going to need to keep the human chromosome that specifies thymidine kinase.

That's why you could follow a whole series. You could just take some hybrids and see what chromosomes are there and what human gene products are there, and then set up a hypothesis that this is on this and this is on that, and then test it specifically by looking at all the hybrids you have that have this chromosome in it and see if that product is –

that's the way it was done at that time. It became an obvious thing that one could do.

JC: How is it done now?

BM: We don't need to do hybrids now. I have *loads* of hybrids in my freezer and I don't know what to do with them because people don't use them the same way. Now you just take a clone probe, and you put it on a metaphase, and you just see what chromosome it belongs to. You don't need to do that. That's what science is all about. Technology keeps making obsolete all the old things that we used to do.

When I started out doing G6PD, A and B variants, we used starch gel [electrophoresis]. It was very heavy and you had to twirl it over a flame to keep it in solution and then pour it and not have any bubbles. It was a time consuming process. People changed the formula of starch. It was starch gel. This was Smithies' marvelous contribution to the field, was starch gel.

Then it was replaced because the starch varieties -- sometimes we'd get starch that worked and sometimes it wouldn't. Then it became more difficult to get the starch that worked. Fortunately, someone developed cellulose acetate electrophoresis, and that was very much smaller and simpler. You could do that and it would take you twenty minutes and you didn't have to shake anything. So nobody needed to know how to do starch gels anymore, but the principle was there.

I've watched all of these technologies sort of go behind because as new things come on, make it easier, you don't have to do that now. Nobody has to clone anything because you just go to the computer and you find the sequence and you construct dimers, and you just get the sequence that you want that way rather than having to have a library and clone everything and do the kind of things that we all – So I've gone through lots of technologies.

I've never been afraid of any of them because I just sort of felt that if your problem takes you there, either it's something you want to continue to keep doing so you learn it yourself, or you collaborate with someone who will do it with you if it's something you don't want to do yourself. So methods are not really problematic, they're sort of helpful. You need to have a good question to ask, and you shouldn't be scared of what kinds of techniques it would take to do them.

Anyway, then we could narrow down the chromosome by using translocations, so you could see it was on the short arm and not the long arm. We spent a lot of time doing that, and I was very pleased that when Jean-Louis Mandel[112] in France isolated the gene for ALD [adrenoleukodystrophy],[113] he said he did it because we'd mapped it there, and that's what the mapping did. We were able to localize it very precisely to a part of the chromosome, and it was small enough for him to try to look for the gene. And he found it. So mapping was very important in those days. People are still mapping, and you have many more markers to use, and you don't need to use hybrids.

JC: I'm wondering if you could tell us about your pasteurization of the human TSIX gene.[114]

BM: Oh, yeah. Well, that's an interesting story, too. We got really inspired by the work that Jeannie Lee[115] and Rudy Jaenisch[116] had done in taking the region of the chromosome that seems to be important and we know is important in initiating the process of X inactivation.[117]

What they did was to take a YAC at the time, which is a yeast artificial chromosome, that contained a rather large piece of this region of the mouse chromosome, and they transfected it into mouse embryonic stem cells. Then when they got it into the cells, they

used those cells to make mice and to study the process in the embryonic stem cells when they induced inactivation. It inserted rather randomly when they transfected it in, so it became a part of an autosome rather than an X chromosome. They put it into a male, who doesn't undergo X inactivation, and they found that when this piece of DNA that was the X inactivation center, presumably, was put into an autosome, it made the mouse think it had two X chromosomes and it started to inactivate the chromosome, even though a male mouse wouldn't do that normally.

So I thought this is a way for us to study – since we can't look at early human embryos and we don't have human embryonic stem cells, we didn't have any at the time that we could look at – that we could use the human region, put it into a mouse and ask whether it would do anything. Now the chance was the mouse wouldn't pay any attention to it because it's human and not mouse. That was one possibility, especially since the gene is not terribly well conserved between the two species. To our surprise, when we did that experiment, in the male mouse, we started X inactivation in that mouse.

It didn't inactivate very much of the region of the chromosome that it inserted into, but it did go a couple of megabases downstream to the thymidine kinase gene on the mouse chromosome. So we did get some effect but not as big an effect as you would if you did a mouse-to-mouse, so that there are some species-specific differences. But the shock was that these sequences were recognized very well by the mouse and enabled it to do that.

Well, we then had this region inserted into mice, and we took the tissues, dissected them out and put them into culture, so we had all kinds of cultures with our transgene in these little mice. They didn't like it, incidentally. I mean, most of them died. They were chimeric,[118] so we got a few of them that could survive. They don't like turning off their only X. (chuckles) It doesn't work very well for them.

So we had these, and we thought, well, let's see if there's anything else that's being transcribed in this region, because we had a good region of four hundred and fifty or so kb [kilobase or 1000 bases] of chromosome, so let's see what else is going on. We have this gene, which was the XIST gene,[119] we knew we had that, but what else is being transcribed from this chromosome in these cells?

We ended up identifying an antisense transcript just downstream of XIST that overlapped the XIST locus, at least at the 3-prime end of the gene. It didn't go all the way. We knew that in the mouse, Jeannie Lee had identified the mouse G6 gene, and it overlapped to the end of the XIST gene to its promoter region, and it seemed to inhibit the transcription of XIST. We went on to further study it and show that it isn't the same, it's undergone different evolution in humans so that it's lost its CpG island in its promoter region. It isn't transcribed from the same chromosome, which would be the one with the active X to turn off XIST in the mouse. In humans it's transcribed along with the XIST gene on the inactive chromosome. I guess we haven't talked about XIST, so you don't know about XIST.

NC: We've read a bit, but it would help to have your –

BM: I was thinking not in terms of you, but I was thinking about anybody who might – d anyway, it's a different gene in human and mice. XIST is the really important gene within the X inactivation center in both mice and in humans and in some other species as well, including cows. It hasn't been found in marsupials. It's a gene that, when it's transcribed at enough of a level, it surrounds the inactive X chromosome and changes its chromatin from active to inactive. Now, some of the details of how it induces this modification are being intensely worked on now, but it does do that. It would do it, as I said, you put it into an autosome, it starts inactivating the autosome, so it doesn't care where it is. It's an RNA

and it's not a protein. It doesn't code for a protein. It's a structural RNA, and it has about seventeen or so exons in mice and eighteen in humans, and that changes, because people discover new ones periodically. It's a large RNA and very potent, very potent.

The story is that if this RNA is allowed to transcribe, it's going to inactivate any chromosome that it's on. But it works only in cis,[120] on the chromosome from which it's transcribed. It's what inactivates all the X chromosomes in a cell. It probably doesn't need any help in inactivating, so the real thing that I'm interested in and working on still is what keeps it from being transcribed on an active X, because you have to have one active X per cell, and something has to stop it from being transcribed on that chromosome.

TSIX may be important in some modulation in the mouse, but it isn't obviously needed for X inactivation in humans. A choice of active and inactive X can't really be decided by an X chromosome, it has to come from somewhere else. I think that this is part of the veils that are added through evolution that complicate the mechanism, where it's really not essential for the basic process.

That's why I like to look at other species, because I don't think you will know what's important in one unless you look at others, because things you may think are terribly important may not occur in the other species, so they sort of divert you from seeing the major factors that are involved. Does that make sense?

NC: Mm-hmm.

BM: OK.

JC: I'd like to jump back to your 1978 book chapter on “Clonal analysis and development: X inactivation and cell communication as a determinant in the female phenotype.”[121] I liked it and I was struck by some of the language, like communication and cooperation and your conclusion saying that X inactivation contributes to the greater biological fitness of females. I was wondering what you had in mind when you wrote the conclusion.

BM: That paper was in '78, yes. I could write the same paper today. I would be able to fill in the details a bit better, but I don't think anything of what I said there would change. The only thing I didn't know of at the time was that you could have mutations that might make a cell do better than the normal allele, and adrenoleukodystrophy is a gene that we learned about subsequent to that, that might maybe modify it and say generally better biological fitness, but not invariably, because in that case, the mutant-type cells have an advantage over the wild-type.

What I was thinking about – and it's on so many different levels, as you may know. Having only a single X chromosome in the male makes him extremely vulnerable to any mutations that affect genes on that chromosome. We, because of X inactivation, have only a single X chromosome in each cell in the same way, but we are mosaic,[122] so we have some cells that have a mutation and other cells that don't. Obviously, having those other cells potentially can ameliorate any effect of the mutation.

Females have an advantage merely because they have the possibility of two populations of X chromosomes. They have only a single cell, but it can be one or the other, and sometimes fifty percent of normal activity in a tissue, because most tissues are mosaic. The patch size is small enough that most tissues, if you take a two millimeter biopsy, you're going to get both tissues represented, both cells, one with the father's X functional and one with the mother's.

We know that males at all ages are more vulnerable to death than females. It's about 1.2. I'm writing about this, and I have a table. I've just been looking at the

population statistics, and the ratio is 1.2 males to every female who dies. When you get to be in the teen age and so forth, it goes much higher than that because there are so many accidents and things that affect males more than females. I'm talking about differences on the first day of life, the first week of life, the first month of life, when obviously differences in experience aren't going to be responsible for death. Then I've been going back in utero, and the same is true post-implantation.

So females have a biological advantage. I mean, there's just no question about it. But they have even more of one because they have – I'll go back. I think part of this advantage has to be due to the fact that males have a single X chromosome. There are a thousand genes on the X, that's the number that's been put forth recently, and they have to do with everything, absolutely everything. There are an awful lot of immunodeficiencies on the X, so if you're going to be more susceptible to infections – and all along the way, you have a better advantage because women who carry genes for immunodeficiencies usually eliminate the cells that have the deficiency, so that certainly puts them at a biologic advantage. They only have the T cells or B cells in some of the disorders that carry the wild-type gene, so that's got to be an advantage over a male, right?

I told you about the Lesch-Nyhan syndrome, where you share goodies from cell to cell so that, through gap junctions, small molecules can pass. Lysosomal enzymes can go from one cell to the other through pinocytosis.[123] So you have a lot of sharing between cell populations and a lot of reasons why female cells are not going to be as deficient as males, and that can't help but be an advantage.

That's what I mean, and I still stand by it. There are some exceptions where you might go the other way. The heterozygotes for adrenoleukodystrophy, because your mutant allele has an advantage, they end up having manifestations of the disease when they get to be thirty, and they're in wheelchairs. So they don't have a severe disorder, most of them don't, in the first decade that kills most of the affected males, but they go on and have manifestations later because of this gradual better survival of the mutant cell.

What I mean is that many genes may have no affect on cell proliferation, but an awful lot of them do. If any mutation – or the nature of even polymorphic alleles – make one cell grow better than the other cell, then you're going to start seeing a preponderance of that other type of cell. That means that it's very dynamic. You either can share, in which case there may not be a selective advantage, or if you can't share these goodies, then one cell will be deficient and the other will not. A very small difference, maybe ten minutes per cell cycle, maybe one minute per – I don't know how long it is, but it will eventually catch up and you will replace one cell population by the other.

You see it, of course, in cancer all the time. That's what happens with cancer cells that have a proliferative advantage. They outgrow the others. Well, it's not so rapid in this situation, but sometimes it can be, depending on what the nature of the mutation is. All of us have mutations of one sort or another, so in general, we do have a biologic advantage. Would you not agree?

IX. Women in Science

JC: What I was wondering was, the 1970s were a very important time in women's history, and the language is strong and it's in a positive light for women, and I was wondering if you had anything in mind beyond the science.

BM: Well, I don't agree with [Harvard President] Larry Summers, if that's what you mean.[124] (laughs) It was very funny. I was at a Cold Spring Harbor meeting shortly after this. It may have been in the early eighties. It was the time when we were cloning X chromosome genes and looking at methylation. There was a meeting on DNA and

structures and things. And there was a group of young women from somewhere who chanted that expression to me. (laughs) They had memorized it. So I think it was sort of a call. (laughs) It was so funny. I hadn't realized that anybody had read it.

NC: Were you in the audience?

BM: No, no. We were on the porch where you eat in the dining hall. It was very funny. I didn't know them necessarily, but they knew me and they had read the paper. I was very pleased that somebody had read it.

Yes, I am a feminist. I believe women can do anything they want to, and I think they're equally endowed at least, and maybe even better endowed than men are genetically. I think there are lots of reasons that women don't do everything they can do. Maybe they don't want to. That's a perfectly legitimate reason as well. But it isn't for lack of ability that women don't do well, I think. Or maybe *they* think they're doing well. Sometimes it just looks too hard out there, and why bother to do certain things?

You need to have someone like my father to push you into something. I would have never been pushed into science at Smith. They thought that what we should do in my era was to marry well and raise a family and help your husband do well. That is what the philosophy was. They told us we should be teachers, that it was a wonderful profession. We got this message. I'm not sure when it came, but it came. We would have chapel and we would have speakers. It was there, that it was wonderful to teach, and the schedule was great. You could be home with your kids in the summer.

Gloria Steinem[125] wrote – she was at Smith five years after me, and she wrote in a commencement address – and I love it, because it was so true. She said that when she thought she wanted to go to law school, they told her to go to Katy Gibbs [Secretarial College in New York] and learn to be a stenographer because that was what she would end up doing anyway. They did ask on our job forms how many words a minute you can type. It was a very important thing.

You were not given the idea that women could be anything themselves, although you could see it in front of you all the time. Women were doing everything, and yet it didn't come through as a philosophy at all. In fact, the [Smith] alumni journal for many years had a column which was called "Reflected Glory," in which all the husbands' attributes were listed. That was the expectation.

I think that you need somebody to be a role model for you or to push you in some kind of way, or to suggest you could be a physicist. I don't think it comes out of something you're born with. I think it's exposure and the ability to have certain experiences that tells you that, ooh, I can do this. I like doing this. If you don't have that, then you're not going to pursue these opportunities. I don't know whether it would be fun to be a physicist. I have no idea. Or an engineer. It isn't my kettle of fish, I think. But I may have been raised differently and I might have thought it was.

So I think it's exposure. Men are expected to do certain things and women are expected to be good moms and supportive people, so that's what they do. It's easier to do what's expected of you, I think.

NC: What's the sex ratio been like in the X inactivation field over the years?

BM: That is interesting. You might have thought that there would be more women involved, but that is not true. There's Mary Lyon and now there's Jeannie Lee and Edith Heard[126] that I know of. Generally, men have been very taken with it. But there are very few women interested in the Y chromosome, I must say that. That is really a man's thing. But I don't think there's been an overabundance interested in X inactivation. I'm not quite

sure that everybody feels the way I do. I think if you're dealing with mice, you don't think of superiority of the female. (laughs)

Most of the work has been – there are not an awful lot of human geneticists who've been involved with X inactivation, because it's hard to approach in human material. You have to use other ways of doing it, like hybrids or mouse ES cells.

When we were looking for TSIX and its expression, I was concerned that we were looking at mouse cells. We were looking at the human gene in mouse cells, but what was really going on in human cells? So we asked John Gearhart if we could – by that time, he did have human embryonic stem cells. With a lot of trouble, we did get the ability to use those cells. It wasn't his problem, but it's all tied up with patents and things of this sort, so you have to promise your life away, or something or other, just to get some cells to work on and that you're not going to publish it without something or other. That kind of thing.

We looked and we were very pleased that yes, they expressed it too. They were cells that had already – the ones that he had that we were looking at were beyond the X inactivation stage, so we were seeing it expressed in – they were five-week to eleven-week-old embryos that were the derivation of his cells that we examined. We had two male and two female lines, and we saw it in the female lines and not in the male lines.

Then I went back to our placentas, which was interesting, human material that we had. I was sort of wondering why it was continuing to be expressed in those cells. In the mouse, it's only expressed for a moment, just before X inactivation, and then it's turned off. But here we had these cells that had already completed X inactivation, the embryonic stem cells, and they were still expressing it.

We went to our placental cells that we had from full-term pregnancies, and lo and behold, they had it too. It's interesting because adults don't express it. Then we looked at some two-month-olds, you know, cells from various stages. And up to about four years you may see it, but then it miraculously gets turned off. I have no idea why. How did we get into that? (chuckles) Oh, women, whether women were involved in –

X. Personal Reflections

NC: Want to ask the Scott Gilbert question?

JC: Sure. We asked Scott Gilbert what he would ask you in an oral history.

BM: Oh, you did? Oh, isn't that funny.

JC: He had several suggestions, but his first suggestion was to take advantage of the fact that she is the dramaturge[127] for our local theater company, and ask you, if you were trying to provide an actress with the motivation to create the role of Barbara Migeon, what would it be?

BM: Oh, isn't that interesting. Scott is so creative and has such a wonderful view. I think you have to think of someone who would have a certain drive. There's something of a driven [character] here. I don't know how an actress would impersonate me, because what is me is not really something that gets on the stage. (laughs) You'd have to see it inside, and I don't know that people could see inside too well.

I can tell you, I don't suffer fools very well, and that's a problem because I probably should be more tolerant of people, but I'm not. I like to see things done well, and I have a lot of motivation. I'm very curious, and I want to know the answers to things. But I don't know how I would tell someone how to act like me. Am I misinterpreting the question? What does he mean?

NC: I think you're getting at it. The way I read it is, how would you characterize your inner self? What is it that drives you, and how would you describe that to somebody?

BM: Well, I don't know. It's very hard. I have these kind of feelings like I know how things should be done, and I know the right way, and I often try and it doesn't work out too well. I've just been doing these postdoc conferences. I know it's the right thing, but everybody's too busy to do them, and I don't know – I'm very frustrated because I don't know how to do it. I just have my idea of how something should be done. It's being suggested to me that I combine it with a clinical conference, and that's not – I can't distinguish how one could do what I wanted to do in the context of that, so I've just been thinking that maybe I ought to have a course. Maybe we could accomplish giving people an opportunity, because we have so many people who don't speak English very well, and they're having to present job interviews. I think we should be helping them in some kind of way.

I like to see things done the right way, and I always admire people who are experimentally aggressive, I like that. I love discussion with people about ideas. It's been very frustrating in the past few years to have less people to talk to about it, because the people who come to my lab have not been graduate students or Ph.D.s. I've had some Ph.D.s from other countries, but it hasn't been quite the same situation. They've been excellent technicians and very good people, but I've had much more fun when I could communicate with people. I love it when Scott and others give me ideas to pursue, because it shouldn't be a one-way street. I like to communicate.

I don't know. She would have to have energy because I do have that. I don't move too slowly. I'm getting slower, but – I just don't know quite how to answer that, but I would like to know the answer to some of the questions that I have been working on. I've been very focused always on one problem essentially.

It's an interesting question because he certainly knows me. (laughs)

JC: Can I ask two more questions?

BM: Yeah.

JC: What has been your biggest battle?

BM: My biggest battle. Well, not being able to have more influence than I've had on how things turned out. I always wonder about that. I think maybe I'm not using language well enough for whatever. I wrote that paper in 1979. It rarely gets referred to, and people write the same paper over and over again. I don't know why they've ignored it and why they – I don't understand exactly that. Maybe if I were taller or (laughs) or more male or something. I have felt along the way it's been sort of hard. I've had some opportunities, I really have. I have certainly influenced study sections that I've been on. And the logic of argument often wins out. I don't know.

For instance, I look at Carol [Greider] and I say, well, she's been very effective. How does she do that? Maybe she's just smarter than I am? Because she's not big (chuckles), and she's just sort of able to – and I admire it very much. But I don't know. That's been frustrating to me to not know quite what it takes to do this.

NC: Barbara, she's a generation later. You made it possible.

BM: Do you think? Well, maybe that's part of it. I was just so excited to see this glass

ceiling leave here. That's been wonderful.

I've been frustrated not to have more of the students that I wanted, but I don't know quite why. I think they would have done – I mean, I see them leave and do patent law, and I think they might have ended up in science if I'd had a chance to work with them. (laughs) You know what I'm saying? So I don't know. Those are the kind of frustrations.

I've had a wonderful marriage and I was lucky to get good kids. All the important things are all there. I'm a professor here, and I have a lovely office, so I really can't complain too much.

One would have liked to have had more attention paid. I don't think my human genetics people understand TSIX at all. It doesn't come into their – I've had this feeling like I've been in the wrong area. If I was working, I probably should have been in biology and not in here. But it's been very important for me to be here. It influences how I think about things. I'm glad I'm here. I wouldn't really want to be in biology. But I think that the ideas I had would have been paid more attention to if I'd been somewhere else. (laughs) I can't tell you where, though.

JC: My last question is, in your career so far, what have you enjoyed the most?

BM: Oh, everything. I've loved it all. I love coming in in the morning not knowing the answer to something and going home knowing about it. I mean, that's one of the highs that I hope I can continue in some kind of way to do without having a lab full of people that can help you do that.

I love teaching young people. I think that's wonderful. I've enjoyed the postdocs here and helping them do their first presentation. I love being there when they give their first talk. That part of it is wonderful.

My colleagues, I do enjoy them. I love Hopkins. It has so many wonderful people. You've always felt there would be somebody to help you if you needed something.

What is it that I haven't enjoyed? I don't really like the administrators that have come in to interfere with things, and the red tape. That part of it I don't care about.

Saul Brusilow,[128] who is one of my colleagues, has said that if you spend thirty percent of your time doing what you really want to do, you should consider yourself extremely fortunate. And I consider myself extremely fortunate. I love continuing to learn. I love going to journal clubs and to good conferences and hearing good science. I don't like hearing what I did today. Somebody gave a seminar at the faculty lunch who wasn't thinking very clearly. So I don't know what you do about that.

I'm so glad that I've done this. My father never understood it, incidentally. He pushed me so much to study medicine. (laughs) He expected me to practice. He never could quite understand what it was I was doing and why I was still in school, essentially. I've been in school all my life, and it's the best. My daughter is an architect. She really likes it, too, so I guess you can get all excited about whatever you do. I think it's wonderful to have something that you can feel passionate about. I feel sorry for people who end up doing something they don't like. This is great.

NC: Great. This is a super place to stop.

Endnotes

[1] Smith College is a private, independent women's liberal arts college located in Northampton, Massachusetts

[2] Stephen Spender (1909-1995) was a leading member of the generation of British poets who came to prominence in the 1930s, a group sometimes referred to as the Oxford Poets. His poems dealt with themes of social justice and class struggle.

[3] Joseph McCarthy (1908-1957), Republican U.S. Senator from the state of Wisconsin, became notorious for his aggressive claims that there were Communists in every branch of the government and in academia, starting a "witch hunt" that lost many people their jobs and forced others into a crisis of conscience.

[4] John B. Stanbury (1915-), a Professor at the Massachusetts Institute of Technology, pursued pioneering research on the diseases of thyroid glands. He is the co-author of *The Metabolic Basis of Inherited Disease.* For an oral history of Dr. Stanbury, see https://www.endocrine.org/~/media/endosociety/Files/About%20Us/Sawin/john-stanbury-031309.pdf.

[5] George Whipple (1878-1976) was an American physician, pathologist, biomedical researcher, and medical school educator and administrator. In 1934, he shared the Nobel Prize for his work on liver therapy in pernicious anemia.

[6] Peter T. Rowley (1929-2006), an eminent American geneticist, chaired the Division of Genetics at the University of Rochester from 1970 until his death.

[7] Meningitis is an inflammation of the protective membranes covering the brain and spinal cord.

[8] Dr. Robert E. Cooke (1920-2014) was head of Pediatrics at Johns Hopkins from 1956-1973. A friend and advisor to Eunice Kennedy Shriver and President Lyndon Johnson, he worked to overcome barriers for developmentally disabled children and was a founder of the Head Start program.

[9] After losing two young sons to rheumatic fever, Baltimore banker Henry Johnston and his wife Harriet Lane bequeathed their joint estate to Johns Hopkins to establish a curative home for ill children and advance the study of pediatric disease. The Harriet Lane Home for Invalid Children opened in 1912 and evolved to become today's Hopkins Children's Center.

[10] Sarcoid is a disease in which abnormal collections of inflammatory cells, or granulomas, form nodules in the lungs and associated lymph glands. Most cases resolve naturally, but a few are life-threatening.

[11] Barton Childs (1916-2010) was Professor of Pediatrics at Johns Hopkins from 1949 until his retirement in 1981 and Professor Emeritus until his death. Among his scientific contributions was the demonstration of random inactivation of X-chromosomes in mammalian female somatic cells. Childs wrote extensively on genetic screening and behavioral genetics and stated in 1999 that health and disease would be understood in the future as based on genetic-environmental interactions. An interview with Dr. Childs is included in this collection.

[12] This congenital form of diabetes is characterized by massive bladder enlargement and bilateral hydroureter and hydronephrosis. Pitressin is an antidiuretic hormone.

[13] The specific gravity of a urine sample indicates the concentration and dilution capabilities of the kidneys.

[14] Bacteria, with a few eucaryotic fungi and protists, are the most numerous and obvious microbial components of the normal mouth and throat flora.

[15] Phage typing detects single strains of bacteria.

[16] BR Migeon, BS Minchew. Some observations on the staphyloccus in a pediatric out-patient population. *Bulletin of the Johns Hopkins Hospital* 1960 Nov;107: 262-270.

[17] Edwards A. Park (1877-1969), the third chief of Pediatrics at Hopkins, 1927-1946, pioneered a holistic approach to pediatric medicine and created divisions of pediatric cardiology, endocrinology, neurology, and psychiatry.

[18] Helen Brooke Taussig (1898-1986) was director of the Harriet Lane Home from 1930 to 1963. She is credited with the conceptual development of the surgical shunt procedure (the Blalock-Taussig-Thomas shunt) used to correct tetralogy of Fallot (the congenital heart defect that is the most common cause of "blue babies.")

[19] H. Bentley Glass (1906-2005) was a pioneering American geneticist. His controversial and

eloquent scientific writing influenced other geneticists even after he had retired. He became Academic Vice-President and Professor of Biological Sciences at the new State University of New York Stony Brook in 1965. His papers are at the American Philosophical Society in Philadelphia. See: http://www.amphilsoc.org/mole .

[20] Glucose-6-phosphate dehydrogenase.

[21] Tijo and Levan established the correct number of human chromosomes (46) in 1955 at the University of Lund in Sweden. See: Tjio JH, Levan A. The chromosome number of man. *Hereditas* 1956; 42: 1-6.

[22] Paul Moorehead, Peter Nowell, et al published their peripheral leukocyte culture methodology in 1960. See: Moorehead PS, Nowell PC, Mellman WJ, Battips DM and Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. *Experimental Cell Research* 1960; 20: 613-616.

[23] Victor McKusick (1921-2008), often considered the founding father of medical genetics, was the University Professor of Medical Genetics at Johns Hopkins until his death, having founded the Division in 1957. He also founded the database of genes and genetic disorders, *Mendelian Inheritance in Man* in 1966 and continued as its chief editor in its print and online forms until his death. An interview with McKusick is available in this collection.

[24] Malcolm A. Ferguson-Smith (1931-) established the first human chromosome diagnostic laboratory in the USA during a fellowship at Hopkins 1959-61. He established the Cambridge Resource Centre for Comparative Genetics in 2002.

[25] Turner Syndrome is a genetic condition in females in which one X chromosome is missing or abnormal. It was first described by endocrinologist Henry Turner in 1938. Girls with Turner syndrome are commonly short, have dysfunctional ovaries and may suffer from a variety of physical disorders (including congenital heart disease and hypothyroidism) and/or cognitive deficits.

[26] Chromatin is the complex of DNA and proteins that forms the chromosomes in the nucleus.

[27] A telomere is the region of repetitive nucleotide sequences at each end of a chromatid [single of a paired chromosome], which protects the ends of the chromosome from deterioration or from fusion with neighboring chromosomes.

[28] Chronic myelogenous leukemia is a cancer of the white blood cells, characterized by increased and unregulated proliferation of the granulocytic cell line in the bone marrow. It is today highly treatable with tyrosine kinase inhibitors.

[29] Haptoglobin is the protein that binds free hemoglobin, thus preventing kidney damage and loss of iron.

[30] Wilma B. Bias (1928-1913) became a Professor of Medical Genetics at Hopkins and founded the Immunogenetics and Transplantation Laboratory there in 1967.

[31] Banding is the staining of bands, or chromosome segments, to allow the precise identification of individual chromosomes or parts of chromosomes.

[32] Torbjorn Oscar Caspersson (1910-1997) was the head of the medical cell research and genetics department of the Karolinska in Stockholm until his retirement in 1977. As a doctoral student there in 1963, he was the first to use an ultraviolet microscope to determine the nucleic acid content of the cell nucleus. In 1969, Caspersson and Lore Zech used a quinacrine mustard stain that revealed light and dark lateral bands along the length of chromosomes. This banding method permitted the accurate identification of all 22 autosomes and the X and Y chromosomes and the highlighting of structural abnormalities and extra chromosomes. See Caspersson T, Zech L, Johansson C and Modest EJ. Identification of human chromosomes by DNA-binding fluorescent agents. *Chromosoma* 1970; 30 (2): 215-227.

[33] Harry Harris [1920-1994] was a pioneering British geneticist. He was one of the first to link variations in protein to variations in DNA and both to medical disorders and abnormalities. Harris spent much of his career at the Galton Laboratory but moved to the University of Pennsylvania as Harnwell University Professor of Medical Genetics in 1976.

[34] Lionel Penrose [1898-1972] was a British psychiatrist, medical geneticist, mathematician and chess theorist who carried out pioneering work on the genetics of mental retardation. He held the Francis Galton Chair at University College London from 1945 to 1965 and influenced many British and American geneticists.

[35] Michael M. Kaback later developed an enzyme assay for prenatal Tay-Sachs screening (see note 36) that enabled prevention and virtual elimination of the disease in susceptible populations. He is currently Professor of Pediatrics and Medical Genetics at the University of California San Diego.
[36] Tay-Sachs disease, first identified in the 1880s, is a rare autosomal recessive genetic disorder, in its most common (infantile) form, causes a progressive deterioration of nerve cells and of mental and physical abilities beginning around six months of age and usually resulting in death by the age of four. The disease is caused by a mutation in the HEXA gene on chromosome 15, resulting in an insufficiency of hexosaminidase A, a vital enzyme that helps break down glycolipids in nervous tissue. Tay-Sachs carriers are found predominantly in Ashkenazi Jewish, French-Canadian and Cajun populations.
[37] Jerry Winkelstein was Division Chief of Pediatrics at Johns Hopkins until his retirement. His major research interest was in the molecular biology of immune deficiency diseases.
[38] Bilirubin is the yellow breakdown product of normal hemoglobin catabolism, excreted in bile and urine. Elevated levels of bilirubin may be indicative of hepatitis or other disorders.
[39] Congenital adrenal hyperplasia refers to a group of autosomal recessive disorders, resulting from genetic mutations that affect the production of cortisol from cholesterol by the adrenal glands.
[40] Mary F. Lyon (1925 -) is a British geneticist, who is best known for her discovery of X-chromosome inactivation, also known as "Lyonization," in 1961. "Lyon's hypothesis" (recognized by EMBO, the European Molecular Biology Organization, as the Lyon Law in 2011) states that only one of the two X-chromosomes is genetically active in female somatic cells; the other is inactivated early in embryonic development. She was head of the Genetics Section of the Medical Research Council's Radiology Unit at Harwell from 1962 to 1987, and continued to regularly work in the lab even after her retirement in 1990. See: Lyon MF, Sex chromatin and gene action in the mammalian X-chromosome. *American Journal of Human Genetics* 1962 Jun; 14: 135 - 148.
[41] As of 2014, www.ergito.com is no longer active.
[42] Harwell in Oxfordshire, originally founded in 1946 as an atomic energy research facility, is today the Harwell Science and Innovation Centre.
[43] Liane B. Russell (1923 -) is an American geneticist who has made extensive studies of the chromosomal basis of sex determination and of the mutagenic effects of radiation and chemicals. From 1947 to 2002, she worked at Oak Ridge National Laboratory in Tennessee, where she eventually became a Senior Fellow. An interview with Dr. Russell is included in this collection.
[44] Japanese-American geneticist Susumu Ohno (1928-2000) worked at the City of Hope Medical Center in Duarte, California, from 1952 until his retirement in 1996. He made important contributions to the understanding of molecular evolution. On his work on the X-chromosome, see: Ohno S Kaplan WD and Kinosita R. Formation of the sex chromatin by a single X-chromosome in liver cells of *Rattus norvegicus*. *Experimental Cell Research* 1959; 18 (2): 415 - 418.
[45] The Barr body, originally identified in 1948 by Canadian researcher Murray L. Barr (1908-1995) is the inactive X chromosome in a female somatic cell. See: Barr ML and Bertram EG. A Morphological Distinction between Neurones of the Male and Female, and the Behaviour of the Nucleolar Satellite during Accelerated Nucleoprotein Synthesis. *Nature* 1948; 163 (4148): 676.
[46] See: Russell LB. Genetics of mammalian sex chromosomes. *Science* 1961 Jun 9;133 (3467):1795 - 1803.
[47] Ronald G. Davidson is Professor Emeritus of Pediatrics and Molecular Genetics at McMaster University in Ontario.
[48] Hemolytic anemia is a condition in which red blood cells are destroyed and removed from the bloodstream before their normal lifespan is over.
[49] Starch gel electrophoresis is a method for separation and analysis of DNA and RNA molecules and their fragments. When an electrical current is passed through a starch gel medium, the molecules are separated by charge and begin to move toward the poles, but are retarded by the starch; the smaller molecules move faster and migrate further, thus allowing separation of the components.
[50] A fibroblast is a connective tissue cell that synthesizes collagen, the structural tissue framework.
[51] A dimer is a macromolecular complex formed by two macromolecules, such as nucleic acids or proteins. A heterodimer is formed by two different macromolecules, while a homodimer consists of

two identical molecules.
[52] SV40 is Simian virus 40, a DNA-based virus found in both monkeys and humans.
[53] Epigenetics is the study of heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence.
[54] Carol Greider is the Daniel Nathans Professor of Molecular Biology and Genetics. She discovered the enzyme telomerase as a graduate student in 1984 and shared the Nobel Prize in 2009 for her research on this enzyme. She and historian Nathaniel Comfort were married in 1993.
[55] Howard Hughes Medical Institute, originally founded in 1953 by the billionaire aircraft designer Howard Hughes, has been run by a group of trustees since 1984. Genetics was chosen as one of the original four research areas at that time. The Institute both conducts its own research at its home campus (Janelia Farm in Virginia) and funds other investigators through grants.
[56] Philip E. Hartman (1927-2003) was professor of biology at Hopkins from 1965. He was a pioneering researcher in microbial genetics.
[57] Now at NIH, with the Genetics of Health and Disease Study Section.
[58] Now Professor of Pediatrics and Genetics at Harvard.
[59] NIH Career Development "K" awards provide early career support for senior postdoctoral fellows or faculty-level candidates.
[60] Cardiologist Richard S. Ross served as Dean of the Johns Hopkins Medical School from 1975 to 1990.
[61] Now Professor and Head of Anatomy and Cell Biology at the University of Iowa.
[62] Director of the Institute of Medical Genetics and Professor of Pediatrics, Ophthalmology, Molecular Biology and Genetics, and Institute of Genetic Medicine at Johns Hopkins.
[63] Professor and Director of Pediatric Urology at Johns Hopkins.
[64] Cystic fibrosis is an autosomal recessive genetic disorder in which thick, sticky mucus builds up in the lungs, digestive tract, and other areas of the body, including the pancreas and liver. CF results from a mutation of the gene that expresses the CFTR protein, which regulates the composition of sweat, mucus and digestive fluids. The most common symptoms are difficulty breathing and frequent lung infections; life expectancy is about 40 years in the US.
[65] Muscular dystrophy refers to a group of inherited muscular diseases characterized by progressive weakness of skeletal muscles, difficulty in locomotion, atrophy and death of muscular cells and tissues, and defects in muscle proteins. There are several different forms and genetic etiologies; life expectancy varies. There are no cures at this time.
[66] Carlo Croce (1944 -) is an Italian-born physician and oncologist whose research focuses on the genetic mechanisms of cancer. He has worked in the US since 1970 and since 2004 has been Director of Human Cancer Genetics and Chairman of Molecular Virology, Immunology and Medical Genetics at the Ohio State University Comprehensive Cancer Center in Columbus. Previously, he had been Director of the Kimmel Cancer Center at Thomas Jefferson Medical College in Philadelphia, where he discovered the role of microRNAs in cancer pathogenesis and growth.
[67] Scott Gilbert is now Howard A. Schneiderman Professor of Biology at Swarthmore College.
[68] Frank Ruddle (1929-2013) was an American geneticist and pioneer in human gene mapping and in chromosome transfer technologies. He joined the Department of Biology at Yale University in 1960 and remained there throughout his career, taking on a joint appointment in Human Genetics when that Department was formed. In 1974, Ruddle organized the first Human Gene Mapping workshop, and in 1980, his lab at Yale created the first transgenic mouse. An interview with Ruddle is available in this collection.
[69] Now Director of the Manton Center for Orphan Disease Research at Boston Children's Hospital.
[70] Daniel Nathans (1928-1999) shared the Nobel Prize in 1976 for his discovery of restriction enzymes that cut DNA segments at specific points, making recombinant DNA technology possible. He was a faculty member in the Johns Hopkins Department of Microbiology from 1962 until his death. His papers and a detailed profile are available at http://profiles.nlm.nih.gov/ps/retrieve/Collection/CID/PD .
[71] Donald D. Brown has worked at the Department of Embryology at the Carnegie Institution of Washington since 1962, serving as Department Director from 1976 to 1994. He has made major contributions to the understanding of gene expression and hormonal control of expression during embryonic development.

[72] Robert M. Blizzard (1936-2005) moved to the University of Virginia in 1973 and worked there until his retirement in 1994. He is noted for his role in elucidating the critical role of growth hormone in child development.
[73] See note 16.
[74] (Under her maiden name Barbara L. Ruben) Rubin MI, Calcagno PL, Ruben BL. Renal excretion of hydrogen ions: A defense against acidosis in premature infants. *Journal of Pediatrics* 1961 Dec; 59: 848-860.
[75] John W. Littlefield (1925 -) was Chair of Pediatrics at Hopkins from 1974 to 1985 and then Chair of Physiology from 1985 until his retirement in 1992. Among his scientific contributions were the discovery of the role of ribosomes in protein synthesis and the development of a method to isolate hybrid cells, which was used by others to localize genes to specific chromosomes and to produce monoclonal antibodies. His papers are in the Hopkins Medical Archives; see: http://www.medicalarchives.jhmi.edu/papers/littlefield.html .
[76] Howard M. Dintzis is now Professor Emeritus of Biophysics and Biophysical Chemistry at Hopkins. His wife Renee Z. Dintzis is Associate Professor of Cell Biology. He carried out a classic experiment in 1961 that determined the linear directionality of protein synthesis (from the amino to the carboxy terminus). See: Dintzis HM. *Proceedings of the National Academy of Sciences of the USA* 1961; 47: 247-261.
[77] William L. Nyhan (1926 -) is currently Professor of Pediatrics at University of California San Diego. He identified Lesch-Nyhan syndrome; see note 83 below.
[78] Joyce Axelman is currently a Laboratory Manager in the Hopkins Translational Tissue Engineering Center.
[79] Trypsinization is the process using trypsin, an enzyme which breaks down proteins, to dissociate adherent cells from the container which they are being cultured.
[80] Gilbert SF, Migeon BR. D-valine as a selective agent for normal human and rodent epithelial cells in culture. *Cell* 1975 May; 5 (1): 11-17.
[81] James D. Watson (1928 -) is best known for his co-discovery of the helical structure of DNA in 1953, for which he shared the Nobel Prize in 1962. He has been one of the leading figures in molecular biology for more than 50 years, serving as Director and then President of the Cold Spring Harbor Laboratory from 1968 until 2007.
[82] Now GIBCO Life Technologies, a subsidiary of Thermo Fisher Scientific; still based in Grand Island, New York; originally founded in 1962 to manufacture serum in horses.
[83] Lesch-Nyhan syndrome is a rare X-linked genetic disorder, in which the mutation causes a deficiency of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT), resulting in an excessive buildup of uric acid in all bodily fluids. The clinical manifestations include moderate intellectual disability, poor muscle control and involuntary movements, including self-mutilation. Treatment is symptomatic and more severely affected individuals will usually die in childhood or adolescence.
[84] See note 77.
[85] Michael Lesch (1939-2008) became a cardiologist and held positions at Harvard, Northwestern University and the Henry Ford Hospital. At the time of his death, he was a Professor at Columbia University and the Chair of the Department of Medicine at St. Luke's Hospital-Roosevelt Medical Center in New York.
[86] Migeon BR, Der Kaloustian VM, Nyhan WL, Yough WJ, and Childs B. X-linked hypoxanthine-guanine phosphoribosyl transferase deficiency: heterozygote has two clonal populations. *Science* 1968; 160 (826): 425-427.
[87] Leonard Hayflick (1928 -) is currently Professor of Anatomy at the University of California, San Francisco, School of Medicine, and was Professor of Medical Microbiology at Stanford University School of Medicine. for the Hayflick limit, see: Hayflick L and Moorhead PS. The serial cultivation of human diploid strains. *Experimental Cell Research* 1961; 25: 585-621.
[88] Thomas R. Hendrix (1920-2014) founded the Department of Gastroenterology at Johns Hopkins in 1957 and served as Chair until 1988. He retired as chairman of the Johns Hopkins Joint Committee on Clinical Investigation in 2001.
[89] Gunter C. Genome biology: She moves in mysterious ways. *Nature* 2005 March 17; 434: 279-280.

[90] Possibly inositol monophosphatase, commonly referred to as IMPase, an enzyme involved in the phosphophatidylinositol [PI] signaling pathway, which affects a wide array of cell functions, including but not limited to, cell growth, apoptosis, secretion, and information processing.
[91] Hypoxanthine-aminopterin-thymidine medium.
[92] Waclaw Szybalski (1921 -), a microbial biochemist, is currently Professor Emeritus of Oncology at the University of Wisconsin – Madison.
[93] Gap junctions are specialized arrays of small channels that permit small molecules and ions to shuttle from one cell to another and thus directly link the cytoplasm of adjacent cells.
[94] Stanley F. Wolf is currently a Senior Scientist at the Genetics Institute in Andover, Massachusetts.
[95] David Schlessinger has been a Senior Investigator and Chief of the Human Genetics Section at the National Institute on Aging since 1997. As Director of the Human Genome Center at Washington University 1987-97, he oversaw the development of the X chromosome map with the concomitant finding of a number of disease-related genes.
[96] Wolf SF, Mareni CE and Migeon BR. Isolation and characterization of cloned DNA sequences that hybridize to the human X chromosome. *Cell* 1980 Aug; 21 (1): 95-102.
[97] CpG islands are regions with a high frequency of CpG sites, that is, regions of DNA where a cytosine nucleotide occurs next to a guanine nucleotide, separated by a single phosphate in the linear sequence of bases. CpG islands are typically 300-3,000 base pairs in length, and have been found in or near approximately 40% of mammalian gene promoter regions. Methylation is the enzyme-catalyzed addition of a methyl group, converting the cytosine to 5-methylcytosine. There is an inverse relationship between CpG methylation and transcriptional activity. Almost no sites in CpG islands are methylated.
[98] Certain chromatin sites are highly sensitive to cleavage by DNase and other nucleases.
[99] Wolf SF, Migeon BR. Clusters of CpG dinucleotides implicated by nuclease hypersensitivity as control elements of housekeeping genes. *Nature* 1985 April 4-10; 314 (6010): 467-469.
[100] Adrian Bird (1947 -) is currently Buchanan Professor of Genetics at the University of Edinburgh, a position he has held since 1990. His laboratory identified the MeCP2 protein, which binds specifically to methylated CpG sites, and showed that disruption of this interaction causes the autism spectrum disorder Rett syndrome.
[101] The TATA box is the core DNA sequence 5'-TATAAA-3' or a variant, usually followed by three or more adenine bases, found in the promoter region. The CAT or CAAT box a distinct pattern of nucleotides with a GGCCAATCT consensus sequence that occurs upstream by 60-100 bases to an initial transcription site signals the binding site for RNA transcription.
[102] Cerebral palsy is a group of conditions in which damage to the motor cortex in the developing brain (up to age 3) results in permanent disability, usually involving movement disorders, but also potentially impairments in sensation, communication and cognition.
[103] Gout is the painful condition caused by over accumulation of uric acid in the joints.
[104] See note 99.
[105] Interleukin is the common name for a group of proteins and signaling molecules that trigger cell growth and proliferation. Interleukins were named when they were originally identified as expressed by white blood cells. They are now known to be expressed by several different cell types.
[106] David Kaslow is now Vice-President of Product Development at Merck.
[107] Weiss MC and Green H. Human-mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes. *Proceedings of the National Academy of Sciences of the USA* 1967; 58 (3): 1104-1111.
[108] The Weiss-Green hybrid cell-line work was done at NYU. Mary C. Weiss is now (as of 2009) at the Institut National de la Santé et de la Recherche Médicale in Paris. Howard Green is Higginson Professor of Cell Biology at Harvard Medical School.
[109] Orlando J. Miller is now Professor Emeritus of Genetics at Wayne State University in Detroit, Michigan.
[110] See: Miller OJ, Allerdice PW, Miller DA, Breg WR and Migeon BR. Human thymidine kinase gene locus: Assignment to chromosome 17 in a hybrid of man and mouse cells. *Science* 1971 Jul 16; 173 (3993): 244-245.

[111] The mitotic spindle is the bipolar structure that segregates the chromosomes during mitosis. The chromosomes are lined up at the spindle equator to ensure their correct orientation and segregation.
[112] Jean-Louis Mandel (1946 -) has been Professor and Chair of Human Genetics at the University of Strasbourg since 2003.
[113] Adrenoleukodystrophy is an X-linked heterogeneous disorder in which very long chain fatty acids accumulate in the body, usually causing a progressive neuropathy and often leading to neurodegenerative decline.
[114] The TSIX or Tsix gene is a non-coding RNA gene that binds XIST or Xist [X-inactive specific transcript], the RNA gene expressed in females by the active X chromosome to inactivate its paired X.
[115] Jeannie T. Lee is currently an investigator at the Howard Hughes Medical Institute and Professor of Genetics and Pathology at Harvard Medical School.
[116] Rudolf Jaenisch (1942 -) is currently Professor of Biology at MIT. He is considered a pioneer in transgenic science, the alteration of the animal genome. Jaenisch has cloned and altered animal cells and human stem cells for therapeutic goals, but he is on record as opposing human reproductive cloning.
[117] See Lee JT and Jaenisch R. The (epi)genetic control of mammalian X-chromosome inactivation. *Current Opinion in Genetics and Development* 1997; 7: 274-280.
[118] A chimera is a single organism (animal or plant) in which different cells have different genotypes, due to development from two or more fertilized eggs (an additional egg or genetic material is added during development).
[119] See note 114.
[120] That is, on genes in the near vicinity; *cis* in Latin is "on this side."
[121] A chapter in Subteiny S and Sussex IM. *The Clonal Basis of Development.* Symposium of the Society for Developmental Biology #36. New York: Academic Press, 1978.
[122] Mosaicism refers two or more populations of cells with different genotypes in a single organism, that have developed from a single fertilized egg (differing from a chimera, see note 118 above).
[123] Pinocytosis is a form of endocytosis (absorption by cells) in which small particles are brought into the cell and then suspended within small vesicles that subsequently fuse with lysosomes, vesicles of internal cellular enzymes that break down the particles.
[124] Lawrence Summers (1954 -), an economist, was President of Harvard 2001-2006, having previously served as Treasury Secretary under President Clinton. In January 2005, he made a speech in which he attributed the low numbers of women in science and engineering to "the variability of aptitude," a statement which probably contributed to his departure from the Presidency the following year.
[125] Gloria Steinem (1934 -) is the internationally known feminist activist and organizer.
[126] Edith Heard (1965 -), a British geneticist, is Chair of Epigenetics and Cellular Memory at the College de France Director of the Genetics and Developmental Biology department at the Institut Curie in Paris.
[127] The dramaturge of a theater company does research on the company repertoire, may assist in developing coherent programs and in casting, editing and staging productions.
[128] Saul W. Brusilow is Professor Emeritus of Pediatrics and Biochemical Genetics at Hopkins. He served as Director of the Division of Pediatric Nephrology 1969-1981 and as Director of the Division of Metabolic Diseases 1981-1998.