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Turning Pages (Nobel Lecture)**

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My fraternal twin, Roger, and I were born prematurely on June 23rd, 1925, in Halifax, England, an industrial town in the West Riding of Yorkshire, although we lived outside Halifax at 2, Woodhall Crescent on Wakefield Road, a row house rented from the town. My father, William Smithies, was at that time working for his father, Fred Smithies, who paid him erratically. My mother, neé Doris Sykes, was a college graduate and taught English at the Halifax Technical College (where she met and fell in love with my father, who was one of her students and younger than she). Not long after our birth, my father found a regularly paying job selling small life insurance policies to local farmers and their families. He was a kind and gentle man with a natural mechanical aptitude that he had inherited or learned from his father. A car was needed for a person selling insurance to scattered customers. So we were unusual in our neighborhood in the 1930s in having one. Not that the car was very special; it was a two-cylinder Jowett and was in constant need of repair. I have vivid memories of "helping" my father, when I was about eight or nine years old, to select the least-worn exhaust valves to use in keeping it running. (The stems of the valves wore badly.)

Our sister, Nancy, was five years younger than us, and a welcome addition to the family. She was a beautiful, fair-skinned, ginger-haired baby, and we five-year-old twins suggested naming her "Buttercup". All three of us were generally healthy and happy, although Nancy would not have survived infected tonsils without the then newly discovered miracle antibiotic drug "Prontosil"—the first of the sulfonamide drugs. I had a similar incident at age seven, but without the Prontosil, and was bedridden for 10 weeks after a bout with "rheumatic fever". This illness left me with what I now know was a trivial mitral valve murmur. However, at that time, the condition was considered serious, and I was not allowed to take part in sports for the next seven years. But in the time that I might otherwise have spent in competitive sports I learned to enjoy reading and making things. And sometime before I was 11, I read a comic strip in which an inventor was the major character. This was what I wanted to be—an inventor! (I didn't know the word "scientist".)

Our mother introduced us joyfully to English literature by reading out loud to us, which she did beautifully, while we waited for my father to come home for the midday meal ("dinner"). Kenneth Grahame's Wind in the Willows *and Lewis Carroll's* Through the Looking Glass *were favorites. And we heard and enjoyed Chaucer's* Canterbury Tales *spoken in middle English. We were often happy when our father was late. A dictionary was a part of our everyday life as children, and continues to this day to be a constant companion in our house.*

The location of the house on Wakefield Road was ideal for children. Behind it was a long oak wood that covered several square miles. In the spring the wood was carpeted with bluebells, and in the fall with acorns. At other times it was a place for children, and lovers. It was also a source of the leaf mold that my maternal grandfather, Ben Sykes, and I collected for his garden. He was a highly intelligent but somewhat short-tempered man who lost his job as a company manager because he could not get on with the son of his employer, who inherited the business when his father died. When I knew him, Grandfather Sykes was working as a paid gardener, which he enjoyed greatly. To keep his mind active, he began learning to speak French at age 70 plus. He enjoyed keeping bees too, and taught our father to love this activity. Later, when father was away in the army, we looked after his bees, and recovered their swarms. Roger kept bees for the rest of his life, and was still harvesting honey from hives that he had in his garden in a London suburb at age 81 shortly before he died.

Across Wakefield Road from our house was a large field from which we twins would help ourselves to rhubarb—illegally, of course. Beyond the rhubarb field were the Calder Valley Canal and the Calder River, both heavily polluted when we lived there—but now recovering well. The Calder Valley was even better for children than the long wood. It had caves in disused quarries; and our childhood girl friends, Margaret and Joan Smith, had a farm on the side of the valley. Above the valley was the village of Norland on the edge of a wild heather-covered moor. This moor was another of our playgrounds, and was where my father took his bees for them to collect the heather honey.

My father must have enjoyed mathematics, because I have a particularly vivid memory of him introducing me to decimals at an early age, writing with his finger on the condensate covering the wall above the bath that I was taking. I even remember the color of the wall as being blue. The same love of mathematics was deeply ingrained in Dr. G. E. ("Oddy") Brown, who later taught me mathematics at Heath Grammar School. He conveyed enough of the logic and principles of mathematics that I didn't need to take any math courses at the University. Indeed, the examiners of my entrance examination to Oxford University commented that my mathematics was "very promising for a person so young." I suspect that they liked the comment I added to my answer to their question "How much does a Spitfire slow down when it fires its 8 machine guns?" Using their data on muzzle velocities, weight of a bullet, rate of firing, mass of aircraft, etc., etc., I calculated that the aircraft would slow down 150 miles per hour. I tried to calculate this again in several ways, but still got the same result. So I added the comment: "I don't believe this result. I think that the correct answer might be around 35 mph."

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I have an equally but quite different vivid childhood memory of being shown, by my Smithies grandfather, how to straighten a bent nail. He, like me, couldn't resist picking up anything that he found lying around because "It might come in useful." This trait was well recognized by Jean Stanier, one of Sandy Ogston's graduate students at the same time as me. Odds and ends of discarded equipment and the like would be set aside and labeled NBGBOKFO—"No bloody good, but okay for Oliver." I still make new devices from what most people would call "junk."

My twin Roger and I went to the school in Copley, a village only a 15 minute walk from our Woodhall Crescent house. Our parents decided to let us go to this unpretentious village school rather than send us to a private school, even though the scholastic levels of the village school were less than desirable. It worked out well. Both of us passed the intelligence test used in 1936 as an entrance examination for acceptance of 11 year olds to a higher level of schooling.

Partly in preparation for this change, we moved to 33, Dudwell Lane, Halifax, a semidetached house that was part of a collection of rather well designed but inexpensive new houses. This house was only a 15 min walk from Heath Grammar School, the school which Roger and I now attended. Shortly after moving to 33, I met Harry Whiteley, the only son of the works manager of a local company that made precision time clocks for factories. Harry's and my interests matched perfectly, and we became and still are close friends. Harry's father had set up in the attic of their house ("the loft") a lathe, a good drill press, and the hand tools needed for making many things. Harry knew how to use them, and the loft became our playground. I had somewhere read about a radio-controlled boat, and we decided to make one. For the transmitter we used a spark coil from a T-model Ford. For the receiver we used a home-made coherer, the same device as the one that Marconi had used in his first wireless telegraphy receiver. This was radio transmission at its basic minimum—and we never got it to work. But, encouraged by my grandfather's commercially made receiver, which used a crystal in place of the notoriously fickle coherer, we progressed to winding our own coils and made a much more up-to-date crystal set that worked well. This in turn led to a one-vacuum-tube radio, which I incorporated into my gas mask case instead of the gas mask that all British children were required to carry in the early days of World War II. Our best radio was a super-heterodyne of an advanced design and had four tubes. It worked as a "bread board", but disappointingly not when rebuilt as a more finished product.

When I was about 16, one of my father's friends gave me the engine from a motorcycle. Harry and I made it run, and became interested in owning a complete motorcycle. My first was a 1926 Rudge Whitworth which was notable for having rim brakes that did not work when it rained. Harry helped me exchange the front wheel for one with a safer internal expansion brake, and I used the Rudge regularly to travel to and from college. I also tried, but to no avail, to make it run on a gasoline–water mixture to eke out the very limited gasoline ration. Subsequently, by judicious trading, I managed to acquire motorcycles of increasing power, but always old, and they were an enjoyable and adventurous part of my life for several years. The cars that succeeded the motorcycles were equally old, and kept up my skills as a mechanic. Modern cars and laboratory equipment are unfortunately now only repairable by replacing subassemblies, so the current generation has lost this strong incentive to learn how to use simple tools.

With a Motorcycle 1949

Heath Grammar School was an Elizabethan free school founded in 1597. When we attended the school, it had a superb staff of dedicated and highly educated teachers. History was taught by C. O. Mackley who tried, in vain, to persuade me to study history with him in the sixth form. Chemistry was the task of A. D. Phoenix—who kept order with the flick of the rubber hose from a Bunsen burner. H. Birchall, the games master, tried kindly to bring me up to speed in athletics, but it was a hopeless task with a boy beginning to play games at age 14. My first year in the sixth form, at age 16, was spent with a few other pupils in supervised study of physics, chemistry, and mathematics at a more advanced level. The first term of my second year in the sixth was spent in unsupervised study in preparation for the Oxford University scholarship exams. I concentrated on physics (I was thinking of studying the subject at the university, although in the end I chose medical school), and was fortunate in being awarded a Brackenbury Scholarship at Balliol College. Consequently, the remaining two terms in the sixth form were a blast in more ways than one. I was allowed to do whatever I wanted to, which was messing around (alone) in the laboratory. I synthesized many substances that caught my fancy, including phenyl isothiocyanate, which my textbook said was one of the worst smelling substances known to mankind. I made nitrocellulose (a constituent of Nobel's smokeless powder), and mercury fulminate (the detonator for his dynamite). Perhaps from some innate cautiousness I did not try to make them explode. Quite the opposite was inadvertently true of the nitrogen tri-iodide that I prepared. I had spilled traces of it which exploded when Mr. Phoenix wiped the bench (he was heard to say in an exasperated and loud voice "Smithies!"). My father had a similar reaction when some that I had put on the top shelf of our living room sideboard exploded with a puff of purple smoke as he walked by; it was extremely sensitive when dry.

I had three remedies for the homesickness that I felt on first going to Oxford. One was to look out of my college room window in the direction of my home in the north of England. Unfortunately I was ac-

tually looking south. I never did get the geography of Oxford right because of this error. The second remedy was to read all the Brontë novels again. The three sisters lived in Haworth, only a few miles from Halifax, and their novels were filled with descriptions of the Yorkshire moors that were such a part of my youth. The third remedy was to go down to the porter's lodge and look for a letter from home. Thereby hangs another tale. Balliol College at that time was heated only by open fireplaces in individual rooms. I lived in a room on the second floor reached by a spiral stone staircase. In the cold, damp weather typical of autumn in Oxford, water would condense on the walls and trickle down the staircase. My room was narrow with ill-fitting windows at either end, and with stones covering half of its floor. It was heated (somewhat) with a small fireplace in which I could burn my weekly ration of coal—it was war time. On one occasion when I returned from my homesick visit to the porter's lodge, the corridor was full of smoke and my fire was gone. I followed the trail of smoke and found two second year medical students enjoying my fire in their grate. We immediately became friends. C. G. A. (Geoffrey) Thomas was one of them—which is how I remember the base-pairing rules of DNA—C with G and A with T.

A. G. "Sandy" Ogston, who had interviewed me during my scholarship exam, was the normal tutor for Balliol college's medical students, but his wartime duties prevented him from being my first tutor. David Whitteridge served in his place. Whitteridge was a brilliant scientist but a hard-nosed tutor. I remember him saying to the Master of Balliol (A. D. Lindsay) during our end-of-term meeting that "Smithies can't spell". Lindsay's response "Oh, all interesting people can't spell," was encouraging. Whitteridge's comments "Diffuse, undisciplined, and at times inaccurate" written across my term paper were typically scathing, but deserved. His verbal comment to another student who had copied part of his weekly essay from a source that Whitteridge could recognize was equally to the point—"These scissors and paste jobs will do you no good." Oxford tutors could be ferocious, but that is what made their lessons unforgettable.

I studied anatomy and physiology with a little organic chemistry for two years as a medical student. I surprised the "real" anatomists and myself by winning the anatomy prize, I think because of my answer to one of the exam topics set by Professor Le Gros Clark, who was a pioneer in what we now call cell biology (he was also famous for uncovering the Piltdown man fraud, and for helping Leakey with his prehuman fossils). I almost walked out of the room on reading the question: "Compare the regenerative powers of muscle, bone and nerve." But I suddenly thought of a principle that I thought made their similarities and differences understandable, and so I stayed. Perhaps Le Gros Clark enjoyed reading my answer as much as I enjoyed writing it.

My third year at Oxford was spent in studying for an honors degree in animal physiology (which included biochemistry). By then Sandy Ogston was back from his wartime duties and had resumed teaching and giving lectures on the application of physical chemistry to biological problems. He was best known for his three-point attachment explanation of how an optically active product can be generated from a symmetric precursor. My weekly tutorials with him were always stimulating and led to many memorable incidents. One occurred during the reading needed to prepare for a tutorial essay on carbohydrate metabolism. After learning something about metabolic pathways, I had been struggling to understand the biological "need" to carry out the complex series of reactions that the body uses to extract energy from carbohydrates. I found the answer in volume 1 of Advances in Enzymology *in a long article written in 1941 by Fritz Lipmann. In this article Lipmann describes the difference between energy-rich and low-energy phosphate bonds, a difference that makes sense out of the complex series of reactions used to metabolize carbohydrate. I read his article in my Balliol college room with a level of excitement that I still remember. I even recollect the look of the glossy paper, the look of the pages, and the color of the cloth binding of the volume—a very similar feeling to that when I was introduced to decimals by my father.*

This introduction to the importance of energy-rich phosphate was the cause of my later coming to Sandy's weekly tutorial with a way to generate an energy-rich phosphate bond from a low-energy phosphoester bond by a cyclical oxidation and reduction scheme. Because my scheme could produce energy for nothing, I knew that it was wrong—like the Spitfire slowing down 150 mph—but I didn't know why. Together, Sandy and I—but mainly Sandy—realized that the standard free energy of a reaction (at that time used to classify the energy resulting from a reaction) was not a valid way of calculating how much energy the reaction would produce within a cell. One needed to know the actual concentrations of reactants and products in order to calculate this. My first scientific paper[1] was the outcome of this endeavor. Looking back at the paper, I can see Ogston's analytical mind at work—the paper hints at what is now known to be correct—the need to keep the reactants within a large molecular complex if realistic rates of reaction are to be achieved. This paper was the first of about half a dozen hypothesis papers that I have attempted over my scientific life.

My college "fire-stealing" friends were masters of how to study with the minimum of effort. We learned histology together by playing a show-and-tell game on Sundays that taught us to recognize the tissue on a microscope slide after only a second's glance—just as one recognizes a face. Once identified in this brief time, one could then carefully describe from memory what should be there. If the slide was of liver, for example, we would say "I can see the stellate cells of von Kupfer etc., etc." We never did see them, but this technique, passed on to subsequent generations, meant that Balliol students always came first in the histology examinations. Organic chemistry was equally conquerable if one used all one's senses, as illustrated by Geoffrey Thomas' finding that all the compounds which we were likely to be given could be identified by three tests: "taste, smell, and appearance". I put his principle to good use in the final practical examination in Biochemistry. On being presented with a clear colorless, slightly viscous liquid that smelled of caramel and tasted acidic, I thought it might be lactic acid. A confirmatory test was positive, and I finished the exam in less than 10 minutes.

Sandy Ogston's fascination with the relevance of physical chemistry to biological systems was infectious, and I decided to drop out of medical school and do research in this field. The fourth and fifth years of my Oxford period were consequently spent in acquiring a sound background in chemistry. Since I already had a first class honors degree in physiology I did not have to worry about how well I would do in the exams. I could therefore pick and choose among the topics that I would study. I had a grand time. My organic chemistry

was confined to biological compounds. My inorganic chemistry could emphasize the simple inorganic materials of biological relevance, Na^+, K^+, F^-, Cl^-, etc., rather than rare earths and the like. And I could emphasize those parts of physical chemistry that I enjoyed or were particularly relevant to biological systems. I remember well studying for and writing what I thought was an outstanding twelve-page essay on "The Pauli exclusion principle and the periodic table", which Ronnie Bell, my first tutor in chemistry, had assigned for one of my early tutorials. I only got half way down its first page when Ronnie spotted a weak link in my argument. The rest of the hour's tutorial was spent in teaching me that "You never, ever, write down anything that you do not understand, or cannot justify."

After completing the undergraduate part of the chemistry degree, and now in my sixth year at Oxford, I joined Sandy's lab in the department of biochemistry as a graduate student. It was a happy place. The oldest of us was Rupert Cecil (a veteran bomber pilot and a wing commander in the Royal Air Force). Rupert, in addition to his own research, managed the complex equipment of the laboratory with complete confidence. One of his responsibilities was a Svedberg ultracentrifuge—a large machine built on a concrete pillar and equipped with a powerful electric oil compressor in a pit below the floor. I never cared for the beast, and studiously avoided being sucked into its tentacles. Nevertheless, my thesis topic centered on an artifactual problem that the ultracentrifuge had generated—"the apparent conversion of the globulin fraction of plasma proteins into the albumin fraction." I was to look for some type of disassociation–reassociation reaction by studying the osmotic pressures of mixtures of proteins. I never did get to that part of my problem, but I had a thoroughly enjoyable two years trying. The outcome was a thesis, half of which was devoted to what are now (to me) un-understandable thermodynamic equations. On later rewriting this part of my thesis for publication I discovered a fatal flaw, so my equations never saw the light of day. The other half was devoted to my development of an extremely precise osmometer. The data it produced were so tight that the line through the experimental points had to be interrupted for them to show. This work was published,[2] although the resulting paper has the dubious distinction of never being cited by me or by anyone else. Nevertheless, this thesis work re-enforced my natural inclination to pursue experiments to a conclusion with little regard for the time required to reach this end.

The osmometer required a home-made water bath with its temperature controlled to within 0.001 °C. This I achieved by using a submerged electric light bulb as a controlled heater. Sandy's next graduate student, Barry Blumberg (Nobel laureate in 1976), inherited my bench—and the water bath. He is said to have destroyed it in a fit of rage induced by the repetitious on–off cycle of its light bulb.

When the time came for me to think about post-doctoral work, Sandy urged me to think about going to the U.S.A. I was not enthusiastic—but was persuaded to overcome my prejudices by Sandy and Robert L. ("Buzz") Baldwin. Buzz was a Rhodes scholar from Madison, Wisconsin, working towards his doctorate with Sandy, and he painted a fine picture of life in Wisconsin. So I applied for and was awarded a Commonwealth Fund fellowship to continue my education as a post-doctoral fellow under the guidance of J. W. (Jack) Williams, a learned physical chemist in the Department of Chemistry at the University of Wisconsin. There were other fine physical chemists in Jack's group, including Bob Alberty, Bob Bock, Dick Golderg, and Lou Gosting. My stay with them increased my knowledge of physical chemistry greatly, but the work I did was not particularly rewarding; it culminated in another article that rightly received virtually no attention.[3] In contrast, the reward from the kindness and collegiality of these colleagues and of the other friends that I made in Wisconsin was enormous. They completely removed my foolish preconceptions about "Americans".

My regard for Americans was further increased by my meeting and becoming engaged to Lois Kitze, a graduate student working in virology. But she was reluctant to cross the Atlantic, as I had earlier been in the reverse direction. So, because my acceptance of a Commonwealth Fund fellowship precluded my staying in the United States, I looked for work in Canada. I was fortunate in finding David A. Scott, who in 1954 offered me a job in Toronto. "Scottie" was an older man when I met him, and was winding down a distinguished career in science. He was the first person to crystallize insulin as a poorly soluble zinc salt, which is widely used in the commercial preparation of insulin and still forms the basis for a slow-release form of the hormone. He was a Fellow of the Royal Society of Canada, and of the Royal Society of England. When I met him, he was working by himself in a small room in the Connaught Medical Research Laboratories, a part of the University of Toronto, and spent his mornings looking for a protein in plasma which he thought might bind insulin. In the afternoons, he played golf. He offered me the opportunity to work on anything I wished "as long as it is related to insulin". After reading the available literature, I chose to look for a precursor to insulin. I never found it. But the difficulties I encountered in trying to find it, and a childhood memory that the starch which my mother used for my father's shirts turned to a jelly when it cooled, led to my invention of starch gel electrophoresis. The high concentration of starch needed to make a strong gel introduced a new variable into electrophoresis—molecular sieving. Finding the best variety of starch and how to process it for making the gels became necessary when my supplier's stock of processed starch was exhausted. Many hours were spent in testing all the raw starches that I could buy, and then in grocery stores finding potatoes from Holland Marsh, New Brunswick, Prince Edward Island, and Idaho from which to make the raw starch. None gave as good gels as those made from my first batch. I eventually found out why: my original supplier had purchased starch processed by a second company that had used raw starch imported by a third company from Denmark because of an attack of potato blight in Canada!

The starch gel method proved very effective. With it I discovered previously unknown differences in the plasma proteins of normal healthy persons, which Norma Ford Walker and I showed were inherited.[4,5] Many new opportunities were opened up, and my friends suggested that I would be helped by having a technician. Somewhat reluctantly I agreed, and was joined part time by Otto Hiller, a young immigrant from Germany. He proved to be an excellent choice. We worked together well and soon became friends. Otto had an excellent mechanical sense, and began to make the starch gel equipment that I and other scientists needed for our work. He came along to Wisconsin when I moved there in 1960, but not as my technician. Instead he set up a business to manufacture the plastic equipment and assemble Heathkit® power supplies which were suitable for the gel electro-

phoresis. He also arranged for a manufacturer in Denmark to produce a starch suitable for making the gels, and then distributed the starch to scientists all over the world.

Otto and I spent many Saturday afternoons in his "shop" doing the same sorts of things that Harry and I had done in the loft. We assembled a Heathkit® digital alarm clock, and found out that it had a design flaw which caused it to lethally "electrocute" its own Intel CMOS integrated circuit. We worked out a remedy after several replacement chips, and had some enjoyable interactions with the Intel engineers who we found had drawn a Mickey Mouse on an unused part of the chip. This led us to try to make our own precision digital clock, and to attempt bread boarding a microcomputer by using Intel chips. But our knowledge and bread-boarding technique proved inadequate. So Otto bought a mail order kit for an Altair 8800 microcomputer, while my interest in making a computer was replaced by using a time-sharing GE computer located in Milwaukee, 60 miles from Madison. Communication was by teletype, and the computer language was BASIC. The immediacy of a time-sharing computer suited me, and I subsequently enjoyed directing my student, Bob Goodfleish, while he wrote a group of programs to extract amino acid sequences from our Edman sequenator.[6] Nearly 10 years later I had the same enjoyment in directing John Devereux during the writing of a group of programs for analyzing nucleic acid sequences. The resulting paper[7] is my most quoted, with >6000 citations. More recently I have returned to devising new biological uses of computers, thanks to the existence of generic programs (such as Stella®) that a person can use for modeling complex biological systems without the help of a computer scientist.[8,9] The greatest value of devising these computer models comes, I have found, from their forcing one to clarify which elements in a complex system are most critical, rather from their ability to replicate experimental data or make predictions.

The discovery of inherited differences in plasma proteins shifted my interests towards genetics. This shift, and my wife Lois' homesickness for the States, led me to return to Madison in 1960 to join the strong genetics group at the University of Wisconsin. But I continued to collaborate with my Toronto friends to decipher the molecular/genetic basis of the protein differences found in plasma. This work revealed how homologous recombination could affect protein structure.[10] It also led me to hypothesize that antibody variability might be achieved by recombination.[11] As a consequence, I had an enjoyable period devoted to protein sequencing with the automatic Edman sequenator.

This protein sequencing period ended with the advent of DNA cloning, which encouraged me to spend a sabbatical year with Fred Blattner on a floor below mine in the Laboratory of Genetics. During this time I learned to handle bacteria, bacteriophages and DNA (and took flying lessons at a small nearby airfield). Fred was deeply involved in developing safe procedures for cloning DNA, which at that time was thought might be environmentally hazardous. One of the safety tests required volunteers, of which Fred and I were two, to drink milk spiked with a large number of bacteria and then determine how many survived passage through the gut. The little packages of fecal material that we had to bring back to the lab were the sources of much merriment. During this period, I was invited to apply for various chairmanships in genetics, biochemistry and immunology. Somewhat selfishly, considering the great contributions that chairpersons can make to the scientific welfare of their faculty and students, I chose to continue my life as a bench scientist. But without this decision I might not have had the time to start the experiments, begun at age 57, which led to my best gene-targeting paper, published after I was 60.[12]

In 1978, Lois and I, by mutual and amicable consent, gave up on our less than ideal marriage. And several years later I followed my mother's example by falling for my post-doctoral student, Nobuyo Maeda. However, we were unable to find a way to continue working together in Wisconsin. So, after more than 25 years, I left Madison to accompany Nobuyo to Chapel Hill, North Carolina, where she had been offered an appointment in the Department of Pathology at the University of North Carolina. Nearly 20 years have passed since that move. We have been happy together, and our science has flourished. The academic environment in Chapel Hill is agreeable and collegiate. The weather changes more gently than in Wisconsin (except for occasional hurricanes), and the winters are less harsh than in the Midwest. As a full-time research professor at UNC I have been able to spend even more time at the bench; and all my experiments using gene targeting to generate animal models of human genetic diseases have been carried out in the nurturing environment of the University of North Carolina.

Photo by Dan Sears.

Music has been a part of my nonscientific life, beginning quite early when, as children, Roger and I both sang in the choir at Copley church. We enjoyed the music and also the camaraderie of boys playing pencil games during the sermons. All three of us children were required by our parents to learn to play the piano from seven until 11, at which time we could choose. Roger chose to learn to play the cello, and he continued playing it and the piano for the rest of his life. Nancy became a professional musician, and taught music in high schools. I stopped music lessons, but continued to sing in the church choir until my voice changed. Later at age 18 during my first year at Oxford I joined the Balliol college choir. In my second year, I auditioned for the Oxford Bach Choir with Sir Hugh Allen—a notoriously brusque conductor, famous for his sharp tongue. He began the audition with a comment and a question "You're from Balliol, I see. This is not your first year, is it?" I agreed. His next question was "Do you know how I know?" I replied "Yes sir, my tie [a Balliol tie] has

been washed." The audition never flagged thereafter, even when he asked me to sing my lowest note, only to be interrupted by his secretary saying "Excuse me, Sir Hugh, but this gentleman is a tenor". To which he responded with "Oh, in that case sing your highest note!" followed shortly thereafter with "Stop! Stop!! You'll blow your head off!!!" I sang with his choir for the remainder of my time at Oxford. And I continued to sing tenor with great pleasure with the Symphony Chorus during both my times in Madison, and with the Mendelssohn Choir in Toronto. In Oxford, I learned to play the flute from an ex-army flute teacher. I was not good enough to play in an orchestra, but I happily played for many years with several small groups and with various accompanists.

My interest in flying also began at an early age, before I was 11. I had read all the "Biggles" books by W. E. Johns—fictional accounts of a World War I fighter pilot. I had also been entranced by the movie serial "Tail Spin Tommy" which played at the Saturday morning "Tuppenny Rush" cinema in Sowerby Bridge, a half hour walk from my home (the admission charge was two pennies). And I had read enough about sailplanes and their instruments to dream of flying them. But World War II broke out when I was 14, and gliding as a sport stopped. It was not until I was 38 that I had my first real encounter with flying. This occurred in 1963 during a visit to Toronto which I had made in order to learn from Gordon Dixon how to sequence proteins. The required experiments did not suit my temperament—so instead I went down to the Toronto Island Airport and spent the next ten days taking flying lessons. Over the course of the next month, now back in the States, I took enough additional lessons at Morey Airport in Middleton, Wisconsin, to be able to solo (fly by oneself). But I did not continue. Not until the late 1970s, when I was 52, was I able to try again, thanks in part to the stimulus to learn new things that is part of taking a sabbatical year. This time, I took glider lessons from "Jake" Miller and power plane lessons from Field Morey. Field, the son of a Lindberg-era pilot, was and still is a world-class flight instructor, and we have had many hours together as student pilot and instructor and many more as friends, including the time in 1980 when I accompanied him as co-pilot on a record-winning flight for a single-engine aircraft across the Atlantic from Goose Bay, Labrador, to Rekjavik, Iceland, and then on to Prestwick, Scotland. We knew it would be difficult because we did not have special fuel tanks. So at the end of the runway at Goose Bay and after being cleared for take off we shut down the engine and topped off the tanks until, after adding several gallons of gasoline, they literally overflowed. After flying for 8.5 hours, we landed at Rekjavik with only three gallons of fuel left, enough to fly for about another 10 minutes! But we beat the previous record—by 17 minutes. Our record held for nearly 20 years.

I learned to fly by instruments with Field, and remember rejoicing with him when "Only one drop dripped" (of sweat from my face). One of my glider students—who, like me, would sweat profusely during instruction—came back from his first solo flight with a big grin on his face, with his hand on the back of his shirt, and with the comment "Look Oliver; it's dry!" Learning to fly is learning to overcome fear with knowledge. This same lesson applies to trying new things in science, and to life in general. I am forever grateful to Field for helping me to learn it, and for giving me the joy of flying airplanes, which still continues after more than 4000 hours of piloting—in all sorts of weather.

Photo from *Midwest Flyer* magazine. Reproduced with permission.

Approaches into airports on cloudy days are carried out with the help of two needles on a dial, from which indirect evidence the pilot can infer the position of the aircraft; if the needles cross at right angles you can infer that you are on the beam. Our first assay for gene targeting was likewise indirect, being based on finding bacteriophages of a specific type; if we found the bacteriophages we could infer that targeting had occurred. The airplane-instrument approach and the gene-targeting experiment both have a moment of truth. When the aircraft comes out of the clouds, either the runway is there, or it is not. Likewise, when DNA from a cell colony identified by the indirect bacteriophage assay is tested directly (by a Southern blot), either the gene is altered or it is not. In 1985, at a Gordon Conference during which I first described our success in gene targeting, I told the audience how I was thinking of this airplane analogy while developing the critical Southern blot autoradiograph. On presenting the positive result to the audience I said "And there's the runway!" All the rest of the speakers at that meeting accompanied their critical data slide with the comment "And there's my runway!"

I am fortunate in having been a bench scientist for almost 60 years, and perhaps somewhat prescient in having kept all my notebooks (of which there are more than 130 since I first began). Together they are a record of my happy life as a scientist. They are also a more or less complete record of the progression and logic of the work that brings me to Stockholm today, and of what I expect to continue when I return to North Carolina. My hope is that in the next 40 minutes or so I can

share this progression with you by TURNING PAGES in these notebooks. And I want to talk to a large degree to the people up in the balconies—the students.

The first group of pages documents my CHANCE invention of molecular sieving electrophoresis. My first job was in Toronto, Canada, and I was looking for a precursor for insulin (which I never found!). In the course of this work, I was having trouble in studying insulin with filter paper electrophoresis, as my January 1st, New Year's Day, 1954 page illustrates. [*"Students, note the day!"*] Insulin stuck to the paper and unrolled like a carpet—the more protein that I used, the further the carpet unrolled (Figure 1, left).

Then, on January 23rd, 1954 (Figure 1, middle) [*"Notice, students, Saturday morning!"*], I learned of a new method of electrophoresis that used a bed of moist starch grains (which do not adsorb proteins) for the electrophoretic medium, instead of moist filter paper.[13] But, in order to find the separated proteins when using this method, it was necessary to carry out a protein assay on each of about 40 slices taken from the moist starch bed. I had no technical help, not even a dishwasher, and I couldn't afford the time to do multiple protein assays for each electrophoresis experiment. Happily, however, when I was a boy I sometimes helped my mother with the laundry, and remembered that the boiled starch she used for my father's shirts set into a jelly when it was cold. This memory suggested to me that I could cook the starch grains, make a gel, carry out the electrophoresis, and then just stain the gel to find the proteins. (Figure 1, right). As a consequence of raids on them when no one else was around, I knew the whereabouts of the best stockrooms in the Connaught Laboratory where I worked, and so I was able to find some starch and test the gel idea that afternoon. [*"Saturday, still!"*] The starch gelled only when its concentration was high, but the result with insulin was, as I recorded in my notebook, "very promising!". I later found out that a high concentration of starch impeded the migration of large proteins more than small proteins. This need to use a high concentration of starch was the chance element in my invention of molecular sieving gel electrophoresis.[14] [*"Molecular sieving occurs, students, when you use polyacrylamide gels with proteins and agarose gels with DNA."*]

Three months later, I tried electrophoresing serum—"just for a rough test"—and next day found a total of 11 components. At that time serum was thought to contain only five components (albumin, alpha 1, alpha 2, beta and gamma globulins), so I knew I was onto something likely to be important. I stopped looking for the insulin precursor, and began to study serum proteins.

Over the next seven months I worked the bugs out of the starch gel electrophoresis method using serum from myself and from two of my graduate student friends at the University of Toronto, Gordon H. Dixon and George E. Connell, whom I co-opted to give blood (Figure 2, left). By the end of October, 1954, I was about ready to publish, when for the first time I ran a sample from a female, Beth Wade (B.W., Figure 2, right).

My notebook entry on that day ("Most odd—many extra components") fails to record that I thought I'd found a new way of telling males from females! Indeed I called one type M, and the other type F, and found this designation to be correct for several male–female comparisons over the next week or so. But, after a hilarious day when one pair of individuals had the M versus F electrophoretic patterns reversed, the gender distinction proved to be incorrect. In its place, I thought it likely that the differences had a genetic basis. So, I contacted Norma Ford Walker, at the Hospital for Sick Children in Toronto. She was a remarkable lady, "one of the founding members of the institutions of human and medical genetics in North America",[15] and together we showed that the differences in the electrophoretic patterns of individuals were determined by common and completely harmless variations in the gene (*Hp*) controlling haptoglobin—the chief hemoglobin binding protein in plasma.[4,5]

We identified three common phenotypes (and genotypes): Hp1-1, (Hp^1/Hp^1), Hp2-1 (Hp^2/Hp^1) and Hp2-2 (Hp^2/Hp^2; Figure 3, left).

This finding opened the next chapter in the book of my scientific life—an OPPORTUNITY to study the genetic differences in proteins, starting with the haptoglobins. This I undertook in collaboration with my ex-graduate student friends, Gordon Dixon and George Connell, who had by then come back to the University of Toronto as junior faculty members.

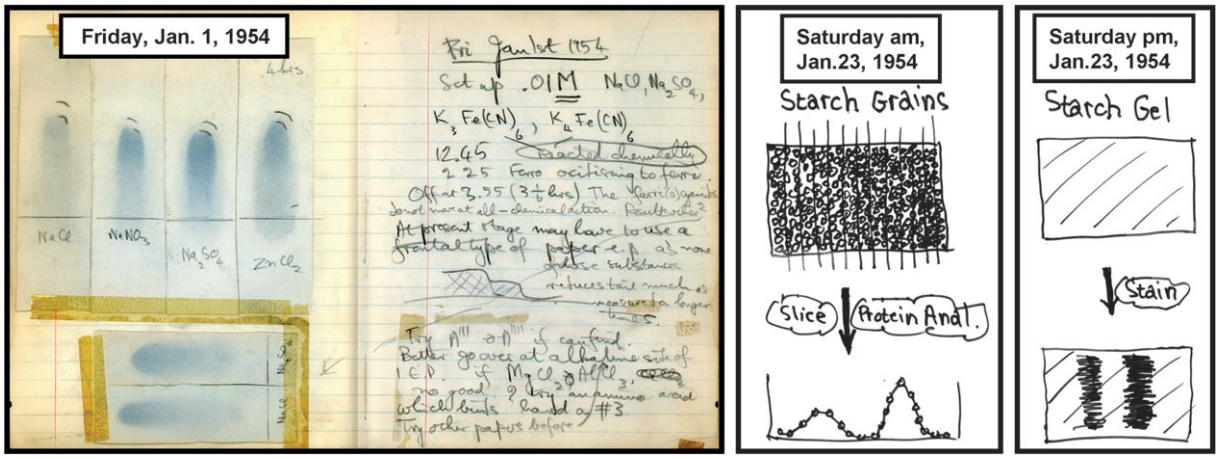

Figure 1.

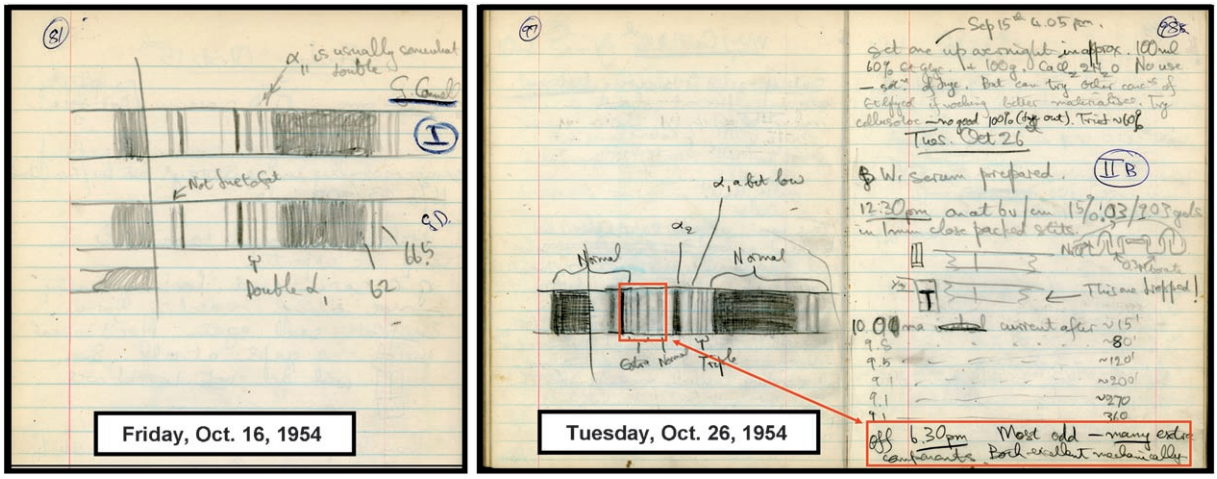

Figure 2.

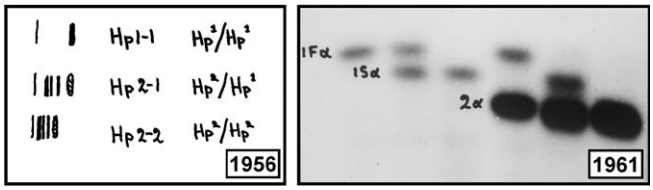

Figure 3.

For many years I have advocated and practiced "Saturday morning" experiments, of which you have already had a sample. These experiments have the advantage of not needing to be completely rational, and can be carried out without weighing chemicals, and so forth. [*"But, students, not without proper lab-book notes."*] And I carried out many of these in trying to simplify the complex electrophoretic patterns associated with the products of the *Hp*^{2} gene. One of them included the use of phenol. This was short-lived because phenol dissolved my apparatus! Reducing the protein with beta-mercaptoethanol (βME) in the presence of urea, following a suggestion from Gordon, proved to be the key. But not without another hilarious incident that followed my accidental breakage of a bottle of βME over my shoes. I put them on the windowsill for a while. But I didn't have many pairs of shoes, and so I soon began to wear them again. Several days later, during a visit for other reasons to the local police station, I heard two old ladies whispering together. One asked the other, "Do you smell it?" Her friend responded, "Yes. Do you think it's a body?" My shoes went outside on the windowsill for a while longer.

After learning how to separate haptoglobin into its subunits (alpha and beta), we found that its genetics were more complicated than Norma Ford Walker and I had thought. Thus, when George began purifying haptoglobin from single bottles of donated plasma, we found (Figure 3, right) that there are three common haptoglobin alleles (*Hp*^{1F}, *Hp*^{1S} and *Hp*^{2}), not two.[16] We also noted that the *Hp*^{2} gene, the one which is associated with the complex protein patterns, appeared to produce twice as much alpha subunit as the other two genes (*Hp*^{1F} and *Hp*^{1S}). And there were other findings that made us think that the *Hp*^{2} gene was more complicated than the *Hp*^{1F} and *Hp*^{1S} genes. For example, when Gordon compared the peptide maps of the hpIFα, hp1Sα, and hp2α haptoglobin subunits, the results were very puzzling, and we had great difficulty in believing them—hp2α appeared to contain all the peptides present in hpIFα and hp1Sα, plus an extra one. Then, during a get together in Toronto in 1961, I remember saying to Gordon and George, "Let's believe our own data." And I suddenly realized that the *Hp*^{2} gene was probably the product of some sort of recombinational event between the *Hp*^{1F} and *Hp*^{1S} genes that had generated a partially duplicated fusion gene. The *Hp*^{2} gene would consequently produce a larger protein having the same peptides as a mixture of hpIFα and hp1Sα together with a novel junction peptide, "J", not present in either hpIFα or hp1Sα (Figure 4, left). We had become the first people to detect non-homologous recombination at the level of a gene! We called it "non-homologous", because the recombination between the *Hp*^{1F} and *Hp*^{1S} genes was within regions that are unrelated in sequence.

We decided to present our data and our partial gene duplication hypothesis at the 1961 Second International Conference of Human Genetics in Rome. We also designed an experimental test that George was going to do before we each gave our part of the story at the conference. He would use the ultracen-

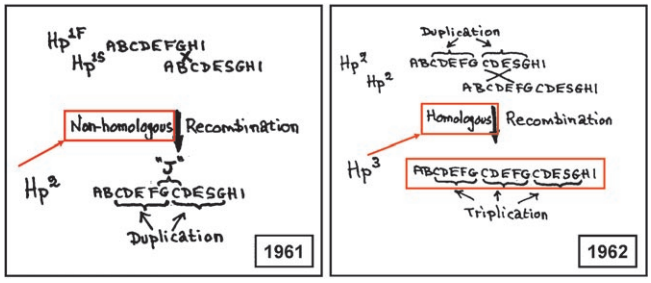

Figure 4.

trifuge to determine the sedimentation coefficients of the alpha subunits with the expectation that the hp2α subunit, which our hypothesis said was larger than hp1Fα and hp1Sα, would sediment more rapidly. We met in Rome on the evening before our talks to review George's results, and he broke the bad news—the sedimentation coefficients of the three hpα subunits did not differ. What to do? Well, we decided, despite this result, to go ahead with our planned talks, with the understanding that in my part of the presentation I would describe our hypothesis and the experimental test of it that we had carried out. Then I would say "We don't believe the result, and I'll go home and invent a new method for determining molecular sizes." The next two pages in my notebooks (Figure 5) show the implementation of that plan.[17] [*"Notice, students, that you shouldn't always believe your results!"*]

The new method showed that hp2α was bigger than hpIFα and hpISα. (Later, when George got rid of aggregation by adding urea, the ultracentrifuge gave the same result.) Together we published our conclusion that the *Hp*^{2} gene was a partial gene duplication resulting from a non-homologous crossing-over event between the *Hp*^{IF} and *Hp*^{IS} genes in a heterozygous individual, *Hp*^{IF}/*Hp*^{IS}.[10]

The next part of this chapter in my science concerns the clear distinction between the randomness of non-homologous recombination and the predictability of homologous recombination. When I told Professor James H. "Jim" Crow, Chairman of Genetics at the University of Wisconsin, about our results, he referred me to some beautiful classical work involving the genes controlling the development of the eye of the fruit fly, *Drosophila*. In succession over a period of over 20 years, Tice (1914),[18] Zeleny (1919),[19] Sturtevant (1925),[20] and Bridges (1936)[21] provided evidence that a unique, non-homologous recombinational event, which occurred only once, had generated a duplication on the X chromosome of the fruit fly that changed the shape of the eye. They also showed that this duplication enabled unequal but homologous recombinational events that occasionally gave rise to a triplication or to a return to the unduplicated chromosome. We extrapolated this result to the haptoglobin genes, and expected that the same type of event would occur with them—namely that unequal but homologous recombination within the duplicated region of the already larger *Hp*^{2} gene would likewise lead repeatedly to a still larger triplicated gene (Figure 4, right). And we found this larger gene as an uncommon variant (*Hp*^{3}, but historically called *Hp*^{2J}) that had arisen independently in all parts of the world where the *Hp*^{2} gene was already in the population. This was my first real understanding of the fundamental difference between the unpredictable nature of non-homologous recombination and the predictability of homologous recombination.

Later, in the late 1970s, I spent a sabbatical period in Fred Blattner's laboratory in the same building as my own laboratory, and learned how to work with DNA and with bacterial and bacteriophage mutants (and, as a concurrent sabbatical activity, learned to fly!). Then, when Fred's Charon bacteriophages were judged to be safe enough for use in cloning human genes, our groups collaborated in isolating and characterizing the two closely related genes that code for the human fetal globins, $^{G}\gamma$ and $^{A}\gamma$.[22,23] Subsequently, when we sequenced these two genes, we found clear evidence that DNA had been exchanged between them as a result of another type of homologous recombination, "gene conversion".[24] So, homologous recombination was very much a part of my scientific ge-

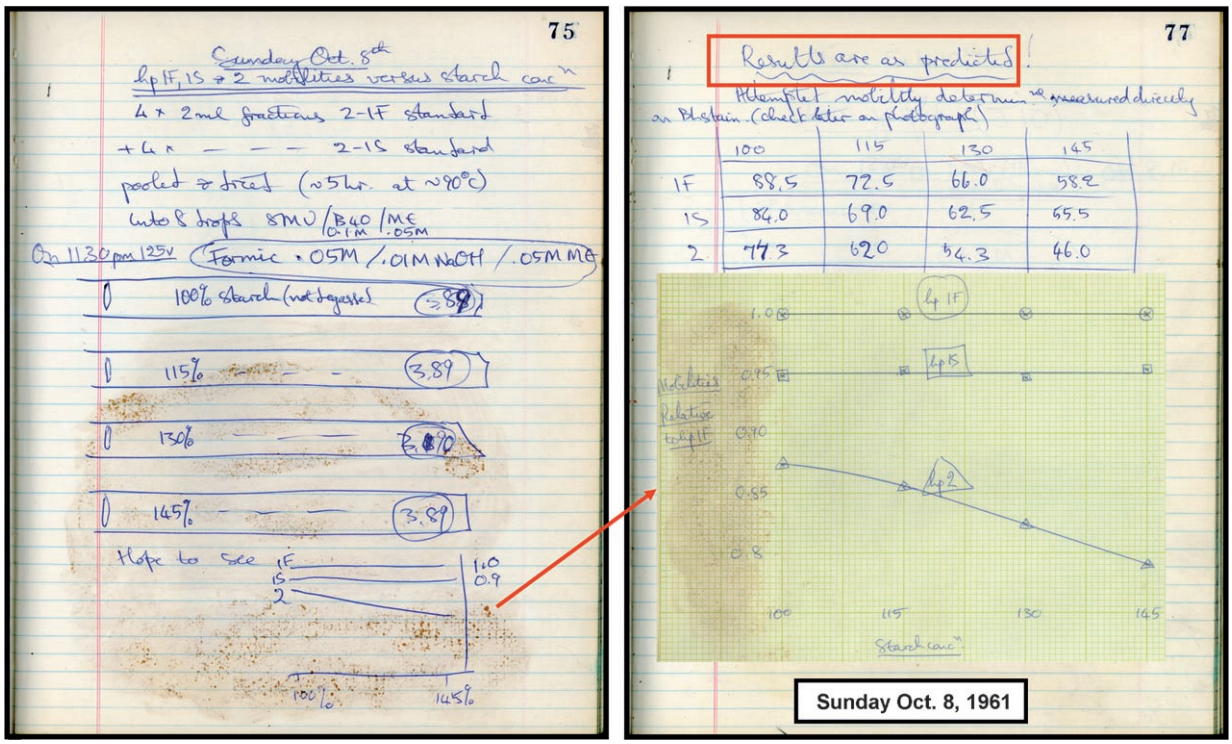

Figure 5.

stalt. And, not surprisingly, having worked with globin genes, I kept thinking that it ought to be possible to use DNA coding for the normal human β globin gene, which was now readily available, to correct the mutant human β globin gene that leads to sickle cell anemia, the most frequent disease caused by a single gene in people of African descent. But no one had demonstrated that such an event (now called "gene targeting") was possible with a genome as large as that of humans and other mammals, although it was known to occur in yeast,[25,26] with a genome of less than one hundredth the size.

Then, in 1982, while teaching a graduate course in molecular genetics at the University of Wisconsin, I came across a beautiful paper that catalyzed me to start writing the next chapter in my book of science—"PLANNING" to use homologous recombination to correct a mutant gene in the human genome. The catalytic paper was published in *Nature* on the 1st of April, 1982.[27] In this paper, the investigators described an elegant gene-rescue procedure to isolate a transforming gene from human T24 bladder carcinoma cells. This gene-rescue procedure depended on using mutant lambda bacteriophages that had a lethal amber chain-termination mutation which could be suppressed if the bacteriophages picked up a copy of *supF* (a mutant tRNA gene able to suppress amber chain-termination mutations). The amber mutant bacteriophages would not grow otherwise. The procedure was complicated, and I had to study the paper carefully in order to use it in teaching. This effort had, however, an unanticipated benefit. During the next three weeks I realized that I could use a modified form of Goldfarb's gene-rescue procedure in an assay to determine whether it was possible to place "corrective DNA in the right place" in the human genome.

On April 22nd, 1982, on page 13 of my γ notebook (Figure 6), I summarized my idea under the heading "Assay for gene placement" (now called "gene targeting"). I proposed to make a DNA construct that included a large fragment of DNA covering the human beta-type globin genes, together with the *supF* gene and the thymidine kinase gene, *TK*. I would then introduce this DNA into human cells that were *TK*$^-$, select for transformants that had become TK$^+$, and then use gene rescue to look for a recombinant fragment in which the *supF* gene was now next to the β-globin gene. This would prove that the incoming DNA had been inserted into the correct place. I was confident that I could detect gene targeting, even if it was extremely rare, because I had three levels of selection: selection in the eukaryotic *TK*$^-$ human cells of transformants that had picked up the *TK* gene and so could grow in a HAT-containing medium; selection in the prokaryotic *E. coli* cells of mutant bacteriophages that could grow because they had picked up DNA fragments containing the *supF* gene; and selection by autoradiography of bacteriophages that also had β-globin sequences. Only homologous recombination could generate the diagnostic recombinant fragment containing both the *supF* gene from the incoming DNA and the β globin gene from the target locus.

At that time DNA sequencers and DNA synthesizers were not yet available, so making the large targeting construct was difficult, and I had to clone it as a cosmid, which I called

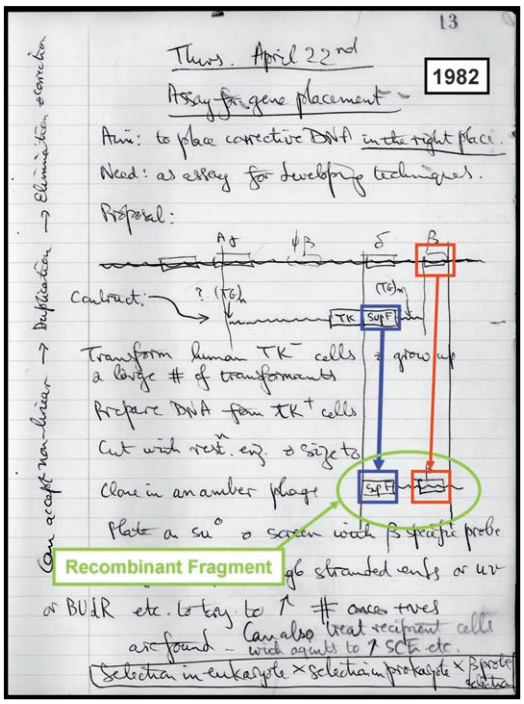

Figure 6.

Cosos 17. Making this cosmid took me seven months. Some idea of the complexity of this task is apparent from the next notebook pages that I show but will not attempt to explain (Figure 7).

By the end of 1982 I had sent Cosos 17 to my collaborator Raju Kucherlapati at the University of Illinois. He was to make a calcium phosphate precipitate with this DNA for transfection into another human bladder carcinoma cell line, EJ. Meanwhile, I began work on what turned out to be a scientifically dangerous experiment: I carried out a plasmid-by-plasmid recombination experiment to test whether the gene-rescue assay would work. The tester plasmid was Δβ17, a small precursor of Cosos 17. The mock target contained the human β-globin gene. The good news was that both the recombination and the bacteriophage gene-rescue assay worked.[28] The unforeseen bad news was that bacteriophages containing the diagnostic recombinant fragment were now present in the lab.

In May of 1983 Raju sent back to us the first DNA sample, RK41, from a gene-targeting experiment with Cosos 17 and the human EJ bladder carcinoma cells. On June 23rd (my 58th birthday), I started the bacteriophage assay phase of this first real test of the overall scheme. 288 bacteriophages grew; 104 (34%) contained some β-globin sequences; but, birthday or not, none hybridized to the critical β globin-IVS2 probe! (Figure 8) So this first real experiment failed to provide any evidence that homologous recombination had occurred.

Over a period of almost a year, my lab and Raju's lab continued experiments with the EJ cells, but without success. These negative results led my graduate student Karen Lyons to suggest that the failure might be because the drug-resistance gene, *Neo*^{R}, which we were now using instead of *TK*, might not be transcribed when incorporated into the β-globin locus of a

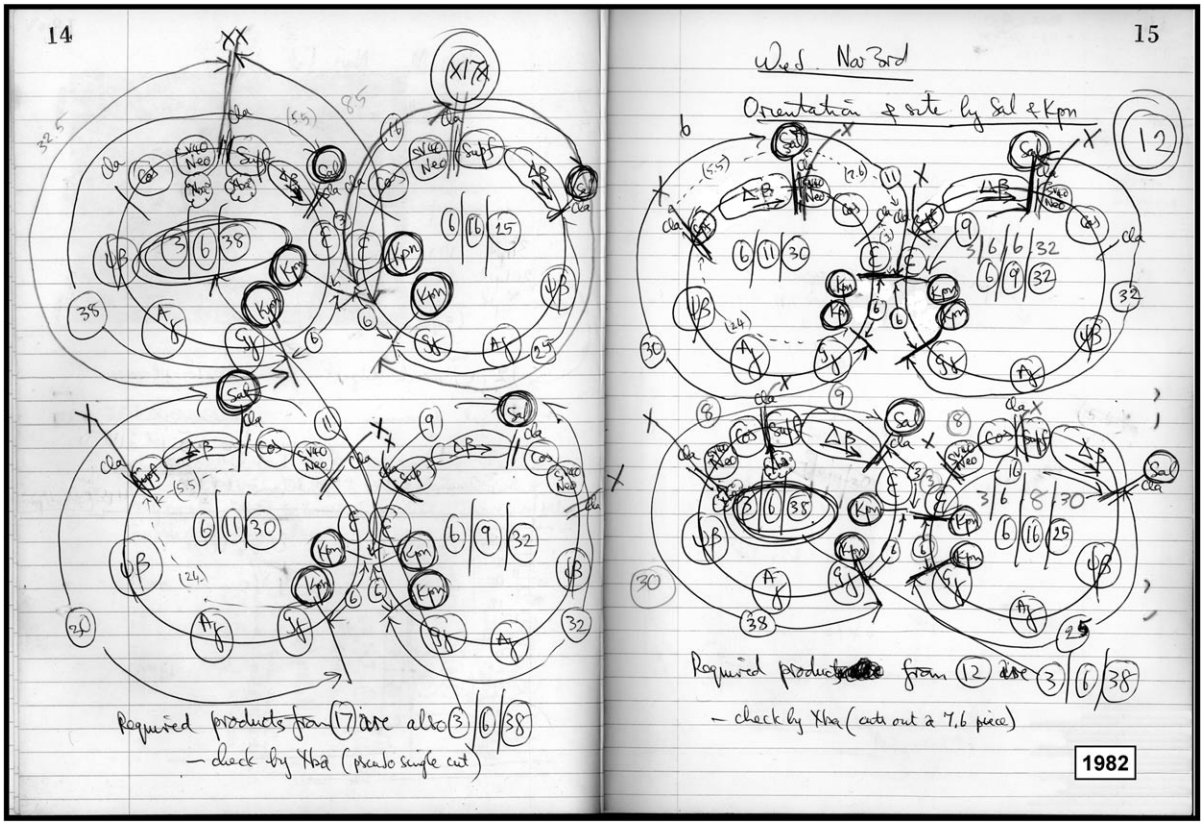

Figure 7.

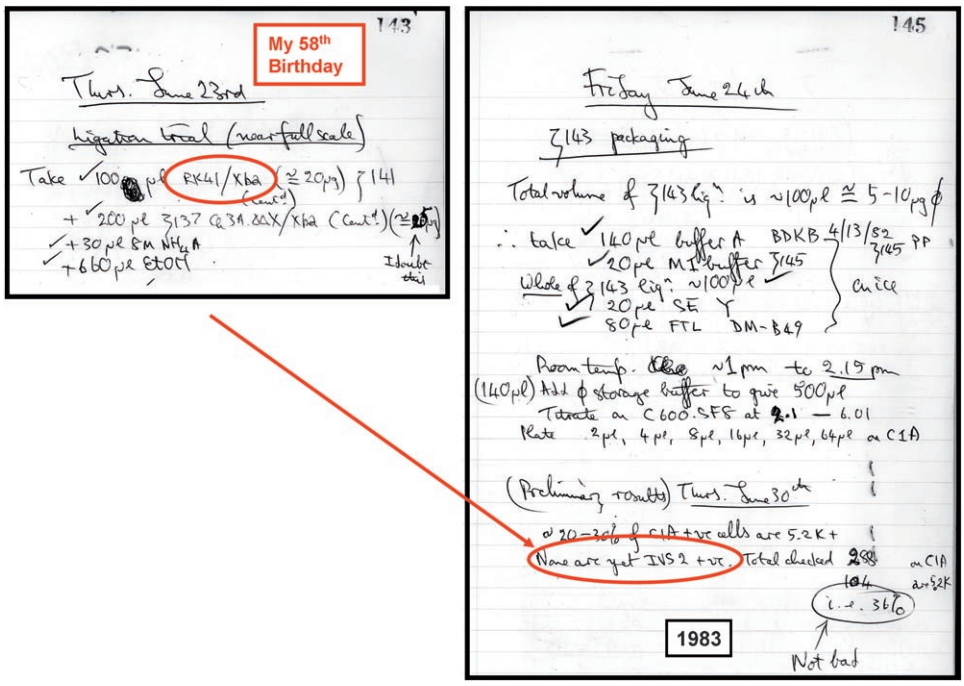

Figure 8.

bladder-related cell that does not express β-globin. [*"Students, you should keep going when things don't work; but you should also think carefully about what might be wrong."*] Two alternatives were available. We could retain the drug selection, but use cells that expressed human β-globin; or we could continue to use the EJ bladder carcinoma cells but without using drug selection. One of our earlier collaborators, Art Skoultchi, gave us a cell line which he had made that was suitable for the first type of experiment. It was a mouse–human hybrid erythroleukemia cell line (which we called Hu11) that carried a human chromosome 11 and expressed human β-globin.[29] Unfortunately the erythroleukemia cells grew in suspension, and could only be transformed by a newly devised procedure—electroporation[30]—and no electroporator was then commercially available. So I spent the next few months designing and testing a homemade apparatus, which was constructed inside a baby bathtub from part of a plastic test tube rack and electronic parts from the local Radio Shack store. The final version of the apparatus, illustrated in

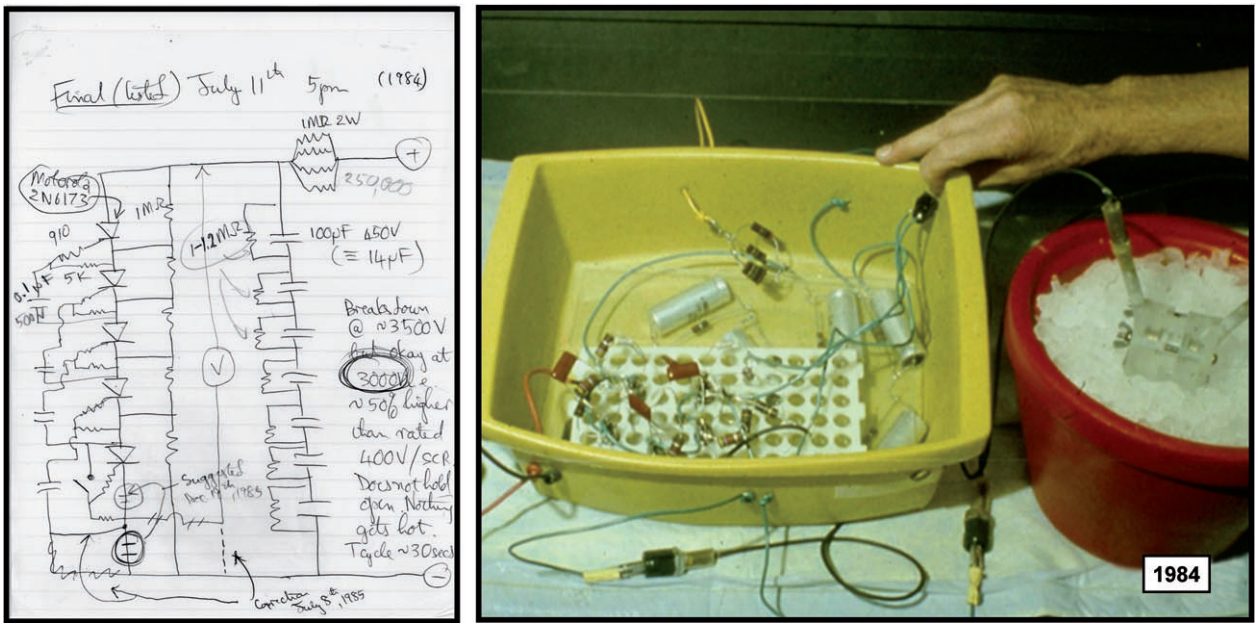

Figure 9.

schematic and real form in Figure 9, does not look impressive—but it worked, and was subsequently used for all the definitive experiments.

[*"Students: never make a complex piece of apparatus that can be bought in order to save money; but by all means make it to save the time that you will have to wait before some manufacturer makes it."*]

Meanwhile Raju did an experiment of the second type, using the EJ bladder carcinoma cells without drug selection. This experiment also used a different targeting construct, Δβ117, illustrated in Figure 10.[28] Δβ117 was the recombination tester plasmid Δβ17 which I had modified so that it could be cut (with *Bst*X I) in the region of homology. This type of cut, we had already shown, increases the frequency of homologous recombination in mammalian cells, as it does in yeast.[31] Raju treated the bladder carcinoma cells with *Bst*X I-digested Δβ117, grew them up without any drug selection, and then sent us DNA from the cells. My technician, Mike Koralewski, tested this DNA with the bacteriophage assay in late August, 1984. He found one IVS2-positive bacteriophage, which I purified and showed to have the hoped-for recombinant DNA fragment with *supF* next to β globin IVS2. This was good news.

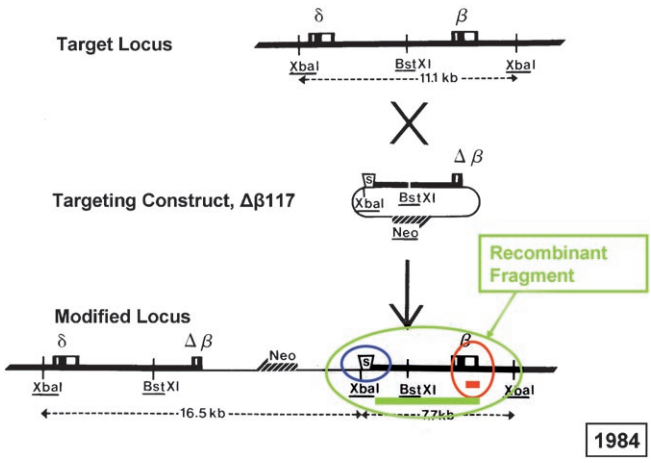

Figure 10.

But we began to have worries. One worry was that this single bacteriophage could have been a contaminant from our recombination tester experiment. (We had had a contamination problem in some earlier gene cloning experiments.) An even more serious worry was that the recombinant fragment present in the bacteriophage might have been formed by recombination in the bacterial cells used in the gene rescue assay, rather than in the mammalian cells used for the transformation. We were discouraged!

Fortunately, however, I had recently bought an airplane, and had flown it to Florida for a short sailing vacation with my pilot friends. This vacation re-energized me sufficiently that I could face starting the Δβ117 experiments again—with two important changes. First, my postdoctoral fellow, Ron Gregg, who had been trying unsuccessfully to inactivate the *Hprt* gene in human fibroblasts, would electroporate *Bst*X I-digested Δβ117 into the Hu11 cells that express the human β globin gene. Second, after Ron had isolated DNA from drug-resistant transfectants, I would digest it with XbaI and size separate the restriction enzyme products into two fractions. One fraction would cover the size range 5.5–8.5 kb, and another would cover the range 8.5–16.5 kb. This fractionation had two purposes. It would reduce the amount of DNA to be packaged into bacteriophages; and, more importantly, it would separate XbaI fragments that were 7.7 kb long (the size of the XbaI recombinant fragment) from any fragments that were 11 kb long (the size of the XbaI fragment from the unaltered target locus). If the recombinant fragment was already present in the DNA

from the Hu11 cells before the DNA had been exposed to bacteria, the 5.5–8.5 kb DNA fraction would give IVS-2-positive bacteriophages. If the recombinant fragment was the result of a recombinational event occurring in the bacteria, the 8.5–16.5 kb DNA fraction would give IVS-2-positive bacteriophages. In early 1985, this fractionation experiment was completed using size-fractionated DNA from a flask containing ~1000 drug-resistant colonies. Two IVS-2-positive phages were obtained with the 5.5–8.5 kb fraction (Figure 11, top). Now we believed our results.

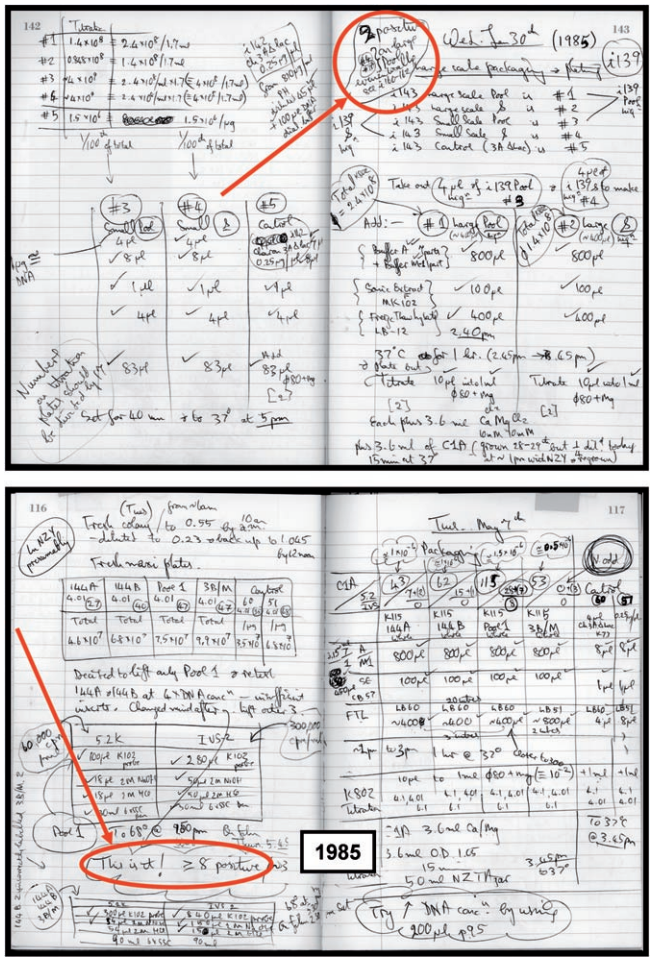

Figure 11.

It took three more months for me to iron out various problems with the gene-rescue assay, and for Ron Gregg to generate pools of individually cloned Hu11 transformants. But by April we had identified a pool of about 300 cloned Hu11 transformants that gave three IVS-2-positive phages. And, in May, DNA from 30 subcloned Hu11 transformants from the 300 pool gave us eight IVS-2-positive phages (Figure 11, bottom). This meant that at least one of the 30 subclones was correctly targeted, and we could now use a direct test for recombination (a Southern blot of DNA from each colony) in place of the indirect bacteriophage assay. On May 18th, 1985 [*"Saturday, yet again!"*], I Southern-blotted Ron's electrophoresis gel of DNA from 11 of these 30 colonies (Figure 12). On May 20th, I noted on page 134 of my κ notebook that subclone "#20 is it!"—three years and one month and seven notebooks after the original idea. In September of 1985, the paper,[12] which I imagine the Nobel Committee considered my most important, was published–after I was 60!

I have already referred to all who contributed to this paper except one—Sallie Boggs. She was a visiting professor from the University of Pittsburgh. She chose, as her part in the work, to ensure that we had a "back-up" to the bacteriophage assay, in case it did not succeed. To implement this, she carried out Southern blots of DNA from 243 individual Hu11 transformants without ever using the phage gene-rescue assay. Although the phage assay, in the end, led to a correctly targeted colony before Sallie found a positive transformant, her work established that the electroporator we had made could introduce single copies of DNA into the cell genome without any other detectable changes in about 80% of transformants.[32]

At this point, it was clear that gene targeting was impractical for any near-term use in the gene therapy that I had initially hoped. The frequency of targeting was too low. The bacteriophage assay we had used to detect targeting was desperate (indeed nobody, including me, ever used the assay again). But these experiments had told us that gene targeting was possible. We now knew that we could introduce DNA into a chosen site and alter a target gene in a preplanned way. So, what to do? Well the first thing was to find a simpler system in which to improve the procedure. And towards this end several investigators in the field independently began experiments with genes that had a directly observable phenotype. Ron Gregg in our group chose the *Hprt* gene, which makes cells resistant to HAT selection when it is normal, and makes them resistant to 6-thioguanine when it is disabled; Mario Capecchi also chose the *Hprt* gene; Raju Kucherlapati chose the *TK* gene. But success was slow in coming.

Then I heard about Martin Evans' work in isolating what we now call embryonic stem (ES) cells and using them to generate mice, and I immediately began to think about using gene targeting in these cells to modify genes in the mouse. Since ES cells grow rapidly and can be cloned from single cells, a low frequency of gene targeting would not be an issue. We could therefore modify a gene in the ES cells, and use the targeted cells to make animal models of human genetic diseases for study and for testing therapeutic procedures. As a step towards this end, in November 1985 Martin personally brought some of his cells to our lab (Figure 13). [*"Students: Don't be shy about asking other scientists for reagents or help!"*]

Martin also put us in touch with Tom Doetschman who had experience with ES cells, which need to be handled correctly if they are to be capable of generating mice. In December of 1987, we published our first use of gene targeting in ES cells—to correct a mutation in the *Hprt* gene of E14TG2a ES cells that had been isolated by Hooper et al.[33] The DNA construct, made by Nobuyo Maeda, worked the first time that Tom used it! The big colonies resulting from gene-corrected cells were easy to distinguish from the tiny residues left from cells in which the mutant gene had not been corrected (Figure 14).

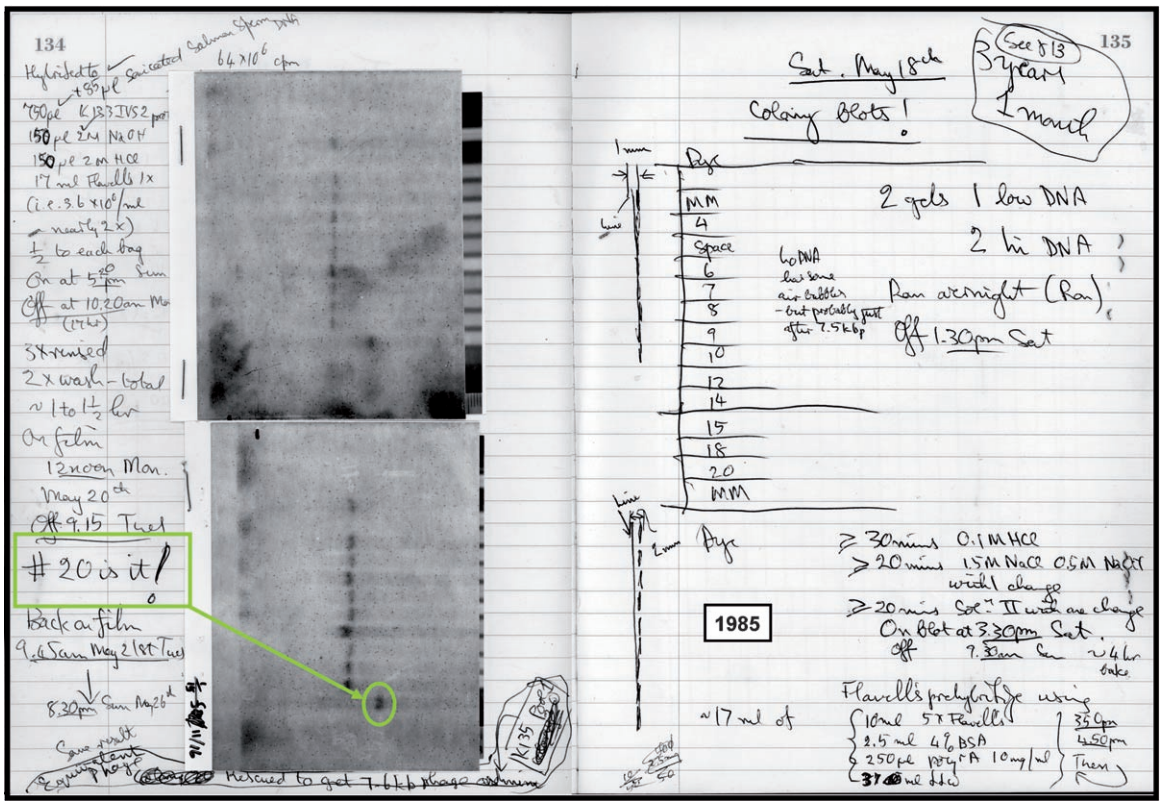

Figure 12.

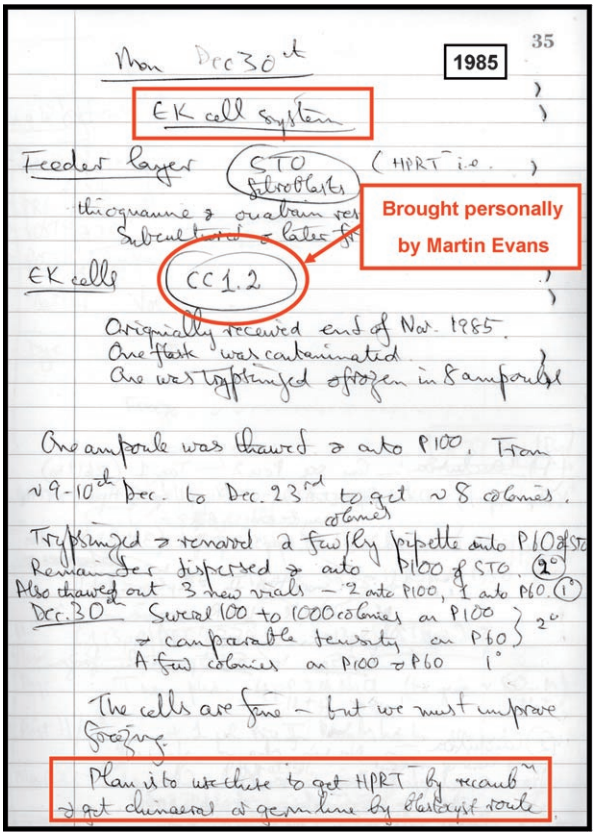

Figure 13.

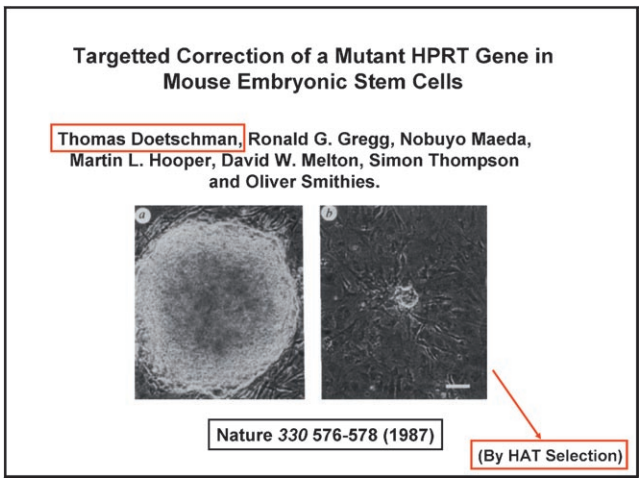

Figure 14.

Mario Capecchi independently contacted Martin Evans for help with ES cells within weeks of our contacting him. And his group's paper, describing a knock out of the normal *Hprt* gene in ES cells,[34] and ours describing correction of a mutant form of the gene,[35] were also within weeks of each other. Both had used drug-selection procedures to isolate the targeted cells, based on the enzymatic activity of HPRT.

However, a procedure was needed for targeting genes that did not have a directly selectable product. A big help would

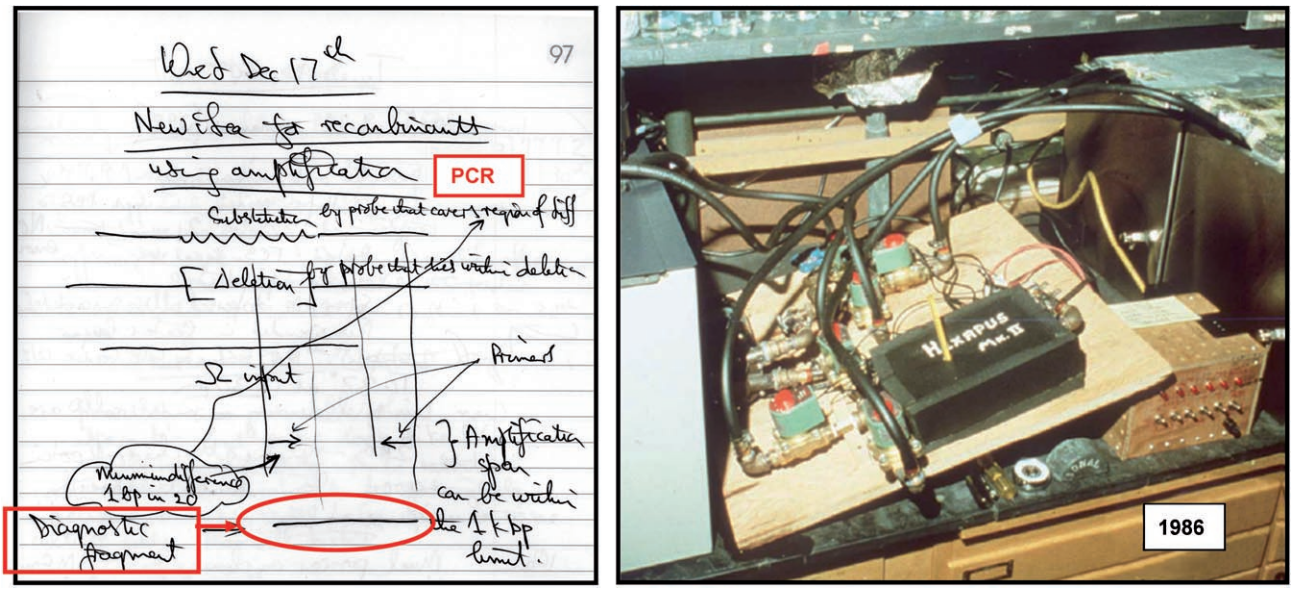

Figure 15.

be to have a simplified recombinant-fragment assay. The polymerase chain reaction (PCR) described by Kary Mullis at Cold Spring Harbor in 1986[36] looked to be eminently suitable for this purpose (Figure 15, left), and I began to work on this idea a few months after hearing Kary talk. Again, no suitable apparatus was commercially available. So Hyung-Suk Kim and I made our own PCR machine, which I still use (Figure 15, right).

Time does not permit me to describe many of the animal models that we have since made using gene targeting in ES cells, with the help of our PCR method of detecting the diagnostic recombinant fragment,[37] together with the powerful positive–negative selection method devised by Mario's group in 1988 as a "general approach for producing mice of any desired genotype".[38] But I can highlight some of them.

Bev Koller, as a post doctoral fellow in my laboratory, was the first to make a mouse model of cystic fibrosis, the most common single gene defect in Caucasians (Figure 16).[39,40]

Nobuyo Maeda and her colleagues made a mouse model of atherosclerosis[41] that became a best-seller at Jackson Laboratories; it is an inspiring model of this genetically complex human disease that causes around 30% of deaths in advanced societies (Figure 17).

John Krege led me into a very productive investigation of genetic factors important in another very common disease—high blood pressure.[42,43] For this work we used a computerized blood-pressure-measuring apparatus made by John Rogers, who was at that time one of my glider pilot students.[44] I chose him to make the new machine (Figure 18) because he had told me about a computerized device that he had designed and built to detect the stones left in pitted cherries, which cause lost teeth in the eaters and lawsuits against the suppliers!

Marshall Edgell helped me to use a different sort of mouse in computer simulations that explored how genetic factors influence blood pressure (Figure 19).[8]

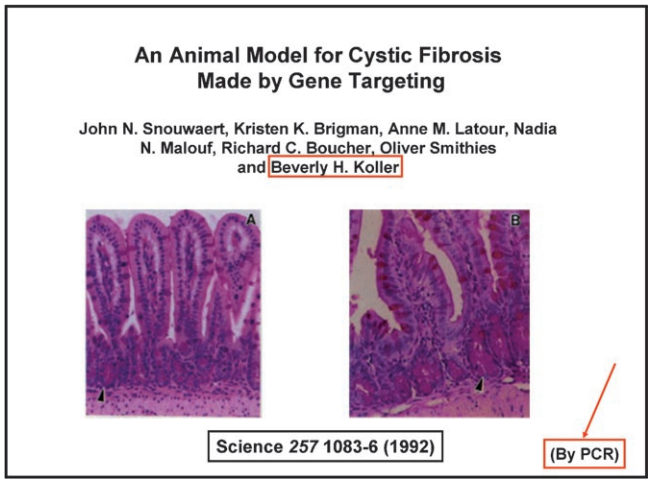

Figure 16.

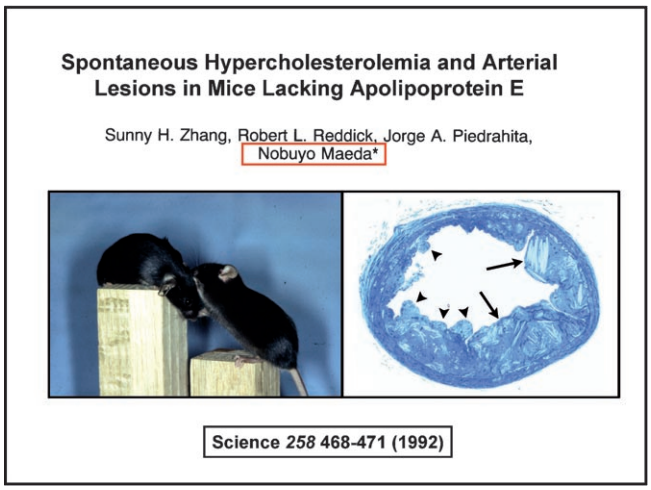

Figure 17.

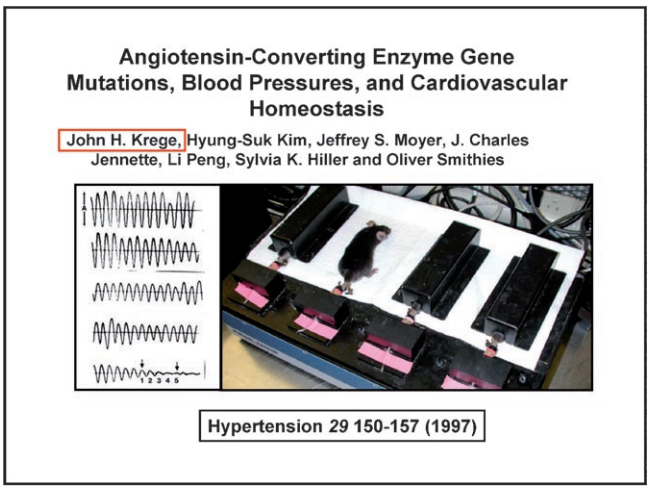

Figure 18.

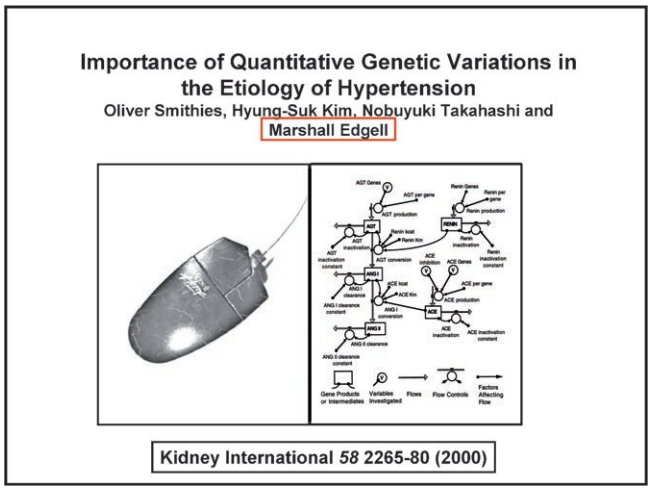

Figure 19.

Devising these and other simulations has helped me to uncover unexpected relationships and has stimulated ideas that I might not have had without this work. In saying this, I stress that the greatest value of these relatively simple computer simulations does not stem from their ability to replicate experimental data, or even make predictions; rather it comes from forcing one to clarify which elements in a complex system are most critical, and how these elements integrate into a logically consistent whole. [*"Students, try a simulation yourself; suitable generic programs for model building are available (for example Stella®) that you can use without being a computer expert"*.]

Before closing, I want to mention a previous visit to the Karolinska Institut on September 6th, 2002. During that visit, I heard Dr. Karl Tryggvason, who is here today, give a fascinating talk on how the kidney separates large molecules from small molecules. But I didn't quite agree with him. And so afterwards, in the corridor, I asked him, "Why doesn't it clog?" His response was, "That's a good question!" which is the one most of us give when we don't have an answer. Suddenly I thought that I already knew the answer, as a result of having recently written a scientific memoir of my undergraduate tutor, thesis advisor, and lifelong friend, A. G. "Sandy" Ogston.[45] In one of

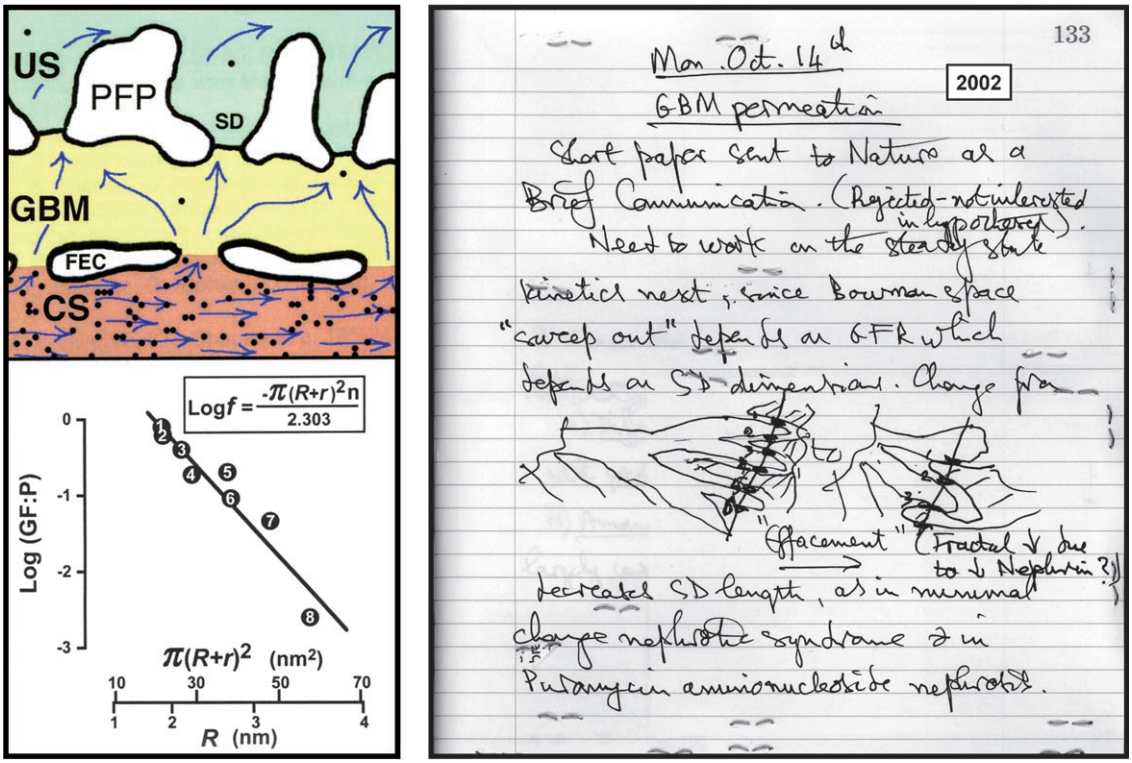

Figure 20.

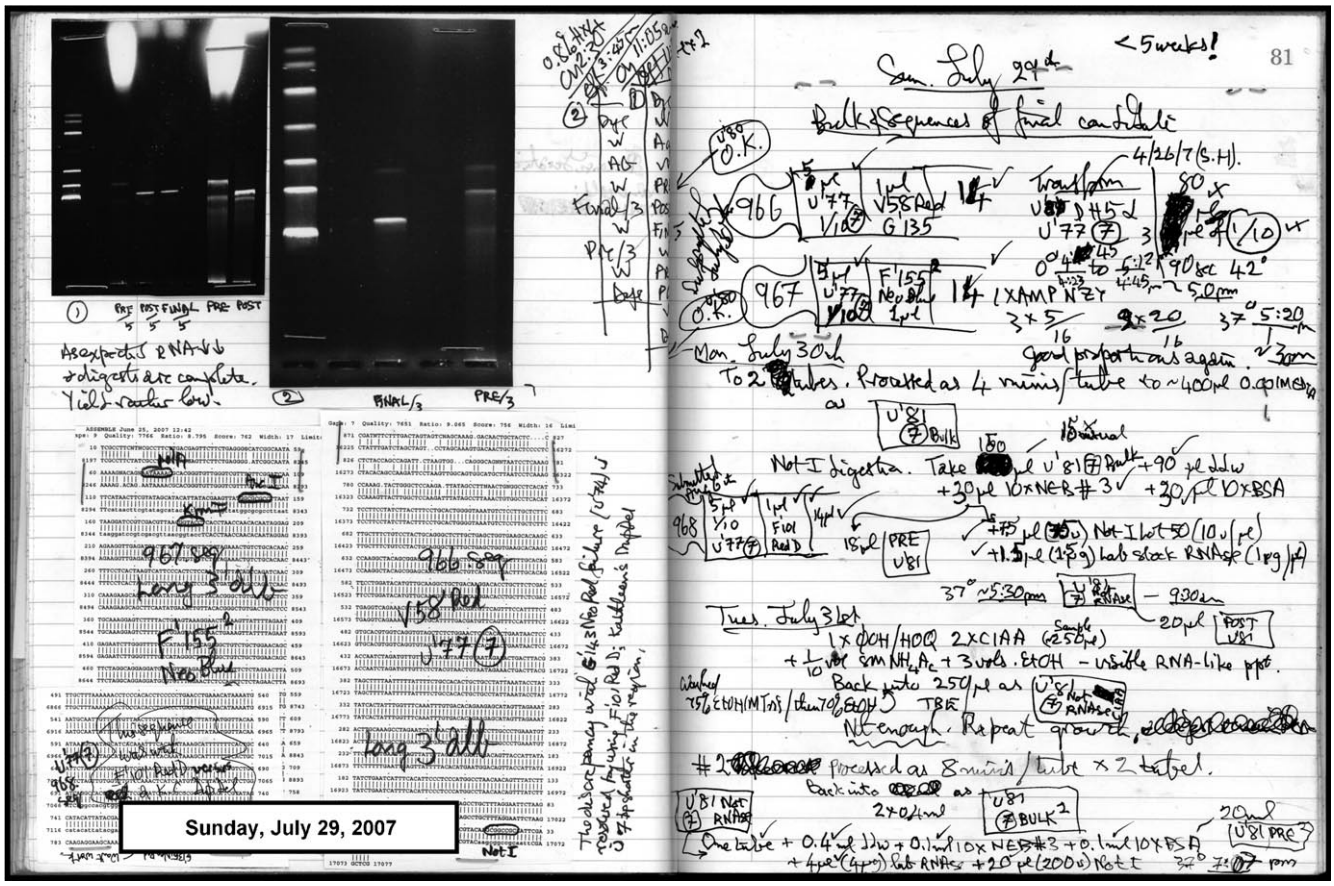

Figure 21.

his papers, Sandy had derived an elegantly simple equation [$f = e^{-\pi(R+r)2n}$] that very accurately describes the behavior in gels of molecules of different sizes.[46] So, on my return to North Carolina, I wrote a brief communication on the topic and sent it to *Nature* (Figure 20).

It was rejected, I'm glad to say, because this caused me to write a better paper that described not only my hypothesis, but also a computer simulation of this aspect of kidney function [*"Another simulation, students!"*], and some testable predictions based on these ideas.[9] My personal scientific efforts are currently directed towards testing the predictions. And the last pages that I turn for you (Figure 21) illustrate the sequencing of a DNA construct made to implement this work.

[*"At 82 it is still possible to work at the weekends!"*]

What's on the next page?

I don't know!!

But that's what makes science exciting!!!

Finally, in closing, I emphasize the importance of choosing a branch of science that makes your everyday work enjoyable, as mine has been. [*"Students: when it was not, I changed it!"*] I also emphasize the importance for a scientist to have other interests for diversion (mine is still flying) when science is being fickle. A happy relationship (mine is with my wife Nobuyo Maeda) can also be a source of comfort at such times—and can provide a captive audience with whom to share science's much less frequent times of bliss. Scientific happiness is in sharing ideas and the daily excitement of new results with students, colleagues, and other scientists. My adviser, Sandy Ogston, had it right when he summarized his view of our discipline. His words are the theme of my visit to Sweden. They capture better than I can what it means to spend a life doing science.[47]

> "For science is more than the search for truth, more than a challenging game, more than a profession. It is a life that a diversity of people lead together, in the closest proximity, a school for social living. We are members one of another."
>
> A. G. Ogston
>
> Australian Biochemical Society Annual Lecture
> August 1970, *Search* Vol. 1, No. 2, 60–63.

Keywords: DNA · embryonic stem cells · gel electrophoresis · gene targeting · Nobel lecture

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