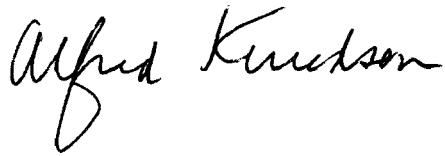

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A Personal Sixty-Year Tour of Genetics and Medicine

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■ **Abstract** The past 60 years surely constitute a Golden Age for biomedical science, and for medical genetics in particular. A personal experience began with an encounter with inborn errors of metabolism, selection, and the incidences of hereditary diseases, and peaked with molecular biology, virology, and cytogenetics, finally focusing all three on the problem of cancer.

If the past dominates my contribution to this volume it is probably because octogenarians have much more past than future. There is also a strong temptation to contemplate a past that spans a Golden Age in genetics from the discovery by Avery and his colleagues that the genetic material of our cells is DNA, to the revelations of the human genome. Here I wish to tell how I first came upon the subject of genetics, how I became diverted to medicine just as medicine was making its first enduring relationship with genetics, and how this merger has led to a preoccupation with medical genetics and the genetic Götterdämmerung that is cancer.

UNEXPECTED BEGINNINGS: GENETICS AND MEDICINE

Had I known when I was born in 1922 that it was the centennial year of the birth of both Francis Galton and Gregor Mendel, it would not have taken me so long to discover genetics. As it turned out, that discovery did not occur until almost 20 years later, when, as a second-year student at Caltech, I took Alfred Sturtevant's course in the biology department of which Thomas Hunt Morgan was chairman and in which Edward B. Lewis was a graduate student. In fact, even biology was new to me because I had never studied it in high school, and I had come to Caltech thinking only mathematics, physics, and chemistry. Of course, each of the last three subjects was part of our education for the first two years, and having Linus Pauling as a teacher in my first year of chemistry and Carl Anderson in my second year of physics, kept those subjects strongly in mind.

The situation changed again because most students enlisted in the Army or the Navy that year and continued our education under government auspices, in uniforms beginning in July 1943. But biology was not a high national priority and I was persuaded to apply to medical school. Of course there was a strong bias in the faculty recommendation that I migrate later that year to Columbia University, the site of Morgan's fly room. Having been mesmerized by Sturtevant's genetics and Albert Tyler's embryology (also a great interest of Morgan's), I was inevitably drawn to pediatrics. I was disappointed in the first two preclinical years because of the excessive memorization required, in great contrast to Caltech's theme of problem solving. To my pleasant surprise, the clinical years brought me back to problem solving.

Despite the fact that I was at a great medical center in New York, as students in 1944 we heard nothing about the discovery by Avery's group across town at the Rockefeller Institute that DNA is genetic material. (Ironically, my inspiring teacher in pediatrics, Hattie Alexander, later demonstrated transformation by DNA in *Haemophilus influenzae* at Columbia.) In fact, there were no classes in genetics, except insofar as some diseases were reported to be hereditary. I did have the accidental fortune to attend a Harvey Lecture off campus, given by George Beadle on his and Edward Tatum's splendid work on the genetic control of biochemical reactions in *Neurospora*, with reference to the earlier work of Garrod, none of which was mentioned in any of our classes. But it was not just the medical school of Columbia (Morgan's university) that overlooked these major advances; there was also no mention of them in any of our textbooks. Indeed, the discipline of medical genetics did not seem to exist 60 years ago.

During my first year of pediatric residency at New York Hospital, I had two memorable experiences. One involved an infant with congenital adrenal hyperplasia whose Addisonian state was being studied by Henry Barnett. Such cases are dramatic, and this one made me curious about its etiology. Later, at Los Angeles Children's Hospital, I saw other cases, one of which had an affected sibling. It was apparent from these cases and reports in the literature that the ratios of affected and unaffected siblings fit a pattern of recessive inheritance. Although I did no biochemistry myself, it seemed that an enzymatic defect in adrenal steroid metabolism could produce too little corticosteroid and too much androgen, the latter causing precocious sexual development in males and pseudohermaphroditism in females, and that the disease could be added to the short list of inborn errors of metabolism that existed in 1951. My first paper brought together my favorite subjects of genetics, biochemistry, and pediatrics (19).

Another influential experience at New York Hospital resulted from the practice of rotating the pediatric residents through the service at Memorial Sloan Kettering Cancer Center, which had no resident pediatricians of its own, although a practicing physician, Dr. Harold Dargeon, who worked there part time, was an enthusiastic student of what we now know as pediatric oncology. His book on the subject was fascinating and the experience of seeing so many children affected with cancer made a deep impression on me. Most exciting was the discovery, there and in

Boston, that new "antifolate" drugs could induce remissions in acute lymphocytic leukemia, a disease for which there had never been a cure before 1950. However, there was no obvious clue that these childhood cancers would later be subjected to genetic study. On the other hand, there was an infant, with huge liver and spleen, afflicted with Niemann-Pick disease, one of the group of genetic sphingolipidoses to which I would return some years later (20). It is interesting that one of these, Gaucher's disease, had been referred to as neoplastic ("epithelioma") by Gaucher himself (11). I still wonder whether there is an increase in numbers of reticuloendothelial cells in these two diseases.

At Los Angeles Children's Hospital I had a genetically like-minded colleague, Dr. George Donnell, who had been stimulated by a case of galactosemia. He and I, with my interest in adrenal hyperplasia, started a genetics clinic and became the self-appointed genetic experts. Unfortunately for me, I left prematurely in 1951 to fulfill an obligation with the U.S. Army as a medical officer during the Korean War, an obligation originating from the fact that many of us were still in medical school when World War II ended, and had not repaid our tuitions with medical service. The Army concluded that a pediatrician could not be helpful in Korea, and I spent my two years at Fort Riley, Kansas, where most of my time was spent in pediatrics, my tasks including the examination of about 1500 newborn infants, one of whom was my second daughter. I feared later that she would envy her two California-born sisters, but she proudly announced to her classmates that *she* was born in an Army fort.

There was no genetics presence at Fort Riley, but I did join Dr. Philip Rothman of Los Angeles by mail in reporting the case of an infant with an "environmental" condition, hypervitaminosis A, who presented with signs of increased intracranial pressure, a symptom we became fascinated with because of the intense headaches in Arctic explorers who ate the livers of polar bears (33). I became very interested in vitamin A, its metabolism, and the effects of vitamin A deficiency. Such deficiency had been a feature of the originally reported cases of cystic fibrosis because this fat-soluble vitamin had been lost from the gastrointestinal tract in that disease. This disease, which had been brought to our attention in medical school by Dr. Dorothy Andersen, a pioneer in its study, had become a major genetic concern in pediatrics. To me it was interesting because it demonstrated how a condition, vitamin A deficiency, could be environmentally or genetically caused. Much later, at the City of Hope Medical Center, I revisited cystic fibrosis, and we demonstrated that, contrary to some opinions, the heterozygote did not show any pulmonary abnormalities (14). We also became interested in its high frequency, presumably caused by some heterozygote advantage that fell historically upon Europeans. We confirmed an Australian report that the grandparents of children with cystic fibrosis had had significantly more live offspring than did controls. The data suggested such great heterozygote selection that the affected populations might not yet be in equilibrium (36).

The subject of heterozygote advantage also arose at the City of Hope Medical Center in connection with patients with one of three genetic sphingolipidoses,

because Gaucher, Niemann-Pick, and Tay-Sachs disease each have an elevated incidence among Ashkenazi Jews. It seemed so unlikely that three metabolically related diseases could all have such incidences in one group that I favored heterozygote selection over genetic drift (25, 30). However, the latter view prevails today. My colleague, George Rouser, was conducting biochemical investigations of our cases, and one of his postdoctoral fellows, John O'Brien, later published his discovery of the enzymatic defect in Tay-Sachs disease (41). Ultimately, the Ashkenazi Jewish population was enabled to engage in population screening for heterozygotes and avoidance of homozygotes to the point that Jewish Tay-Sachs cases have essentially disappeared in our country (18), demonstrating the power of the rational use of medical genetics. This extreme outcome could in theory be realized for cystic fibrosis and the major hemoglobinopathies.

THE REVOLUTION IN BIOLOGY

Between my years at Fort Riley and the City of Hope Medical Center, I returned to Caltech because the world of science was passing me by and I felt a great need to correct this deficit. I wrote to Beadle, Morgan's successor in biology there, who miraculously arranged for me to do so. Little did I know what would be there. It was 1953, and a few months later Jim Watson would arrive, joining such greats as Max Delbruck, Renato Dulbecco, Lewis, Roger Sperry, and, of course, Beadle. Imagine a department of 20 or so faculty members that included six future Nobel Prize winners! With my interest in inborn errors of metabolism, I decided to concentrate on biochemistry and genetics for the next three years with Henry Borsook as my adviser for the former and Ray Owen, who should have shared in a Nobel Prize for his pioneering work on immune tolerance (42), as my adviser in genetics and teacher of immunology. Borsook had produced an important paper in 1935 (3) on the idea of protein turnover, involving continuous synthesis and destruction and requiring energy, providing indirect evidence for such a process. It was only later that Schoenheimer, working with Urey and the new tool of deuterium as a marker, was able to demonstrate this directly. In his great book, *The Dynamic State of the Body Constituents* (46), his first reference is to Borsook's work. Borsook was then investigating hemoglobin synthesis, and he and Jacques Kruh demonstrated such synthesis in reticulocytes, which of course have no DNA (38). How could a cell with no genes synthesize a specific protein? Somewhat later, Richard Schweet, who had moved from Borsook's laboratory to the City of Hope, demonstrated the cell-free synthesis of hemoglobin (47). The isolation of messenger RNA was around the corner, and its association with ribosomes was soon demonstrated for both bacteriophage-specific RNA and host-specific RNA (4, 13).

In the process of studying histidine metabolism, I also had the wonderful experience of meeting some 15–20 other graduate students, including several whose paths I would cross over future years: Dale Kaiser, Bruce Ames, Robert Metzenberg, Leonard Herzenberg, Gordon Sato, and Marcel Baluda. It was truly an exciting time, and as busy as life can become, enhanced as it was for me by

three young daughters. It was surprising to me that, after I had been there three years, it was difficult to find a position in academic pediatrics; genetics had not yet become a significant discipline in academic medicine in 1956.

Fortunately, I did find a position at the City of Hope Medical Center, as head of a then small department of pediatrics; there, for six years, I would care for children with cancer and selected genetic diseases, especially the sphingolipidoses. For the first time I focused my attention primarily on the problem of cancer, including its treatment and its biology. One project involved a study of potential etiologic factors in 100 cases of childhood leukemia, whereupon it became apparent that there was no single factor. Radiation did emerge, as did genetic predisposition (21). There were also three children with Down syndrome, reflecting an already known relationship. I discussed one of these cases with Jerome Lejeune in late 1958, during a visit he made to see Susumu Ohno at the City of Hope. He told me that he had some new information about Down syndrome that would be published soon that might help explain the relationship, but he did not relay that information to me. A few months later I noticed his name on a paper with the minimally informative title "Etude des chromosomes somatiques de neuf enfants mongoliens" (39); this was the discovery of trisomy in that disease! It was, of course, the first report of a chromosomal aberration in a human disease, and the beginning of medical cytogenetics.

There followed a flurry of papers on chromosomal abnormalities in human disease, especially congenital defects and cancer, the latter because it was long known that cancer cells usually show multiple karyotypic abnormalities. However, in 1960, Nowell & Hungerford (40) discovered a new kind of alteration, e.g., a single small "Philadelphia" chromosome in the leukemic cells of patients with chronic granulocytic (or myelocytic) leukemia. This was the first specific aberration in a specific cancer, and it pointed strongly toward the idea that genetic change might in some cases be the cause rather than the result of cancer. For me it was a great stimulus to study cancer genetics.

However, there was another theory about cancer that was thriving at about the same time. It had been known from studies of avian myeloblastosis (8) and Rous sarcoma (45) that viruses (AMV and RSV) could cause cancer, but such investigations required a new approach to their in vitro analysis, which was supplied by Dulbecco and his colleagues at Caltech. I had the fortune to take his virology course at Caltech, and to have one of his former students, Marcel Baluda, as a colleague at the City of Hope. Our search for a human leukemia virus in cells from my patients was unsuccessful, but working with Baluda on AMV, we demonstrated that, as for RSV, this RNA virus required DNA synthesis to replicate (28). This phenomenon was later explained by viral production of reverse transcriptase (1, 50). Baluda subsequently provided evidence that host DNA contains a sequence of DNA that is complementary to AMV RNA. This conclusion was firmly substantiated for the *SRC* gene of RSV by Stehelin et al.'s (49) discovery of the protooncogene. Meanwhile, I became interested in the possibility that human cancer might be transmitted by an integrated tumor virus (23), whose survival in the host could be selected for by its provision of protection by interference with a pathogenic virus. Ray Owen

and I later speculated that tolerance of a virus could protect against disease much as the sickle cell hemoglobin mutation provides heterozygote advantage against malaria (32).

My 10 years at the City of Hope coincided closely with an explosion of molecular biology following the work of Watson and Crick. During these years, I left clinical medicine to become chairman of a new biology department there, with Baluda and Ohno as two of its members, along with William Kaplan and Robert Seecof, both *Drosophila* geneticists, and Jesse Sisken, a cell biologist. Interest in tumor viruses was one reason for my move, but another was the exciting work of Jacob & Monod (17) on enzyme induction and the regulation of gene expression, which opened the understanding of the tissue specificity of gene action. Another reason was the beautiful work on the structure of hemoglobin, and of the relationship between specific mutations and disease mechanism, first disclosed by Hunt & Ingram's (16) discovery of the amino acid substitution in sickle cell anemia following Pauling's and his colleagues' (43) proposal of that condition as the first "molecular disease."

I became so interested in these developments that I readily accepted an invitation to write a book on medical genetics that would recount these great accomplishments and their contribution to understanding mechanisms of disease. The result was a small volume, *Genetics and Disease* (22), with just eight chapters, the first three on genes, chromosomes, and disease, with consideration of mutation and selection. Fortunately, H.J. Muller was visiting, and working at, the City of Hope for one year, and he kindly read and commented on this part of my manuscript. He signed his communications "Joe," but all of us found it difficult to call him anything but Professor Muller. Next were two chapters, one on inborn errors of metabolism with Beadle and Tatum, and Jacob and Monod figuring prominently, and one on molecular genetics and disease and the story of messenger RNA. Nearly every reference in this chapter is from the startling decade of 1953–63. I concluded with three chapters whose progress was not as complete, but had high relevance to common disease; the subjects were infection and immunity, cancer, and diseases associated with aging. From this I developed a new perspective on cancer as a disease to be investigated.

In the 1960s I had another experience that caught me up in the excitement of this new world of genetics. From 1964 to 1968 I served on the NIH Genetics Study Section. This involved much effort (about 70 reviews per year), but it was remarkably rewarding because we had a clear view of new work both proposed and in progress. We were still in a time when all genetics research, from bacteriophage to humans, was reviewed by a single study section. Not only was this a great educational opportunity, but I shared the duties with such stars as Tatum, James Crow, Lewis, Alan Campbell, Gus Doermann, Marcus Rhodes, Clark Cockerham, Margery Shaw, Eldon Sutton, Barton Childs, and Jack Schull. In a way the real star was the executive secretary of our study section, Katherine Wilson, a thoughtful geneticist who seemed to know all of the priority scores even before we met.

RETINOBLASTOMA: A LESSON FROM PEDIATRICS

Writing my book and encountering all of the exciting research of this period caused me, and other members of the Genetics Study Section, to think about medical education and its failure to incorporate genetics. At the time, Bentley Glass was involved in starting a new medical school on the campus of the State University of New York at Stony Brook, on Long Island. It seemed like such an exciting opportunity to have an impact on medical education that I gladly accepted a position there along with Edmund Pellegrino. It was a wonderful experience in many ways, but it became apparent that the world of medicine was not ready to assign a central role to genetics. I abandoned the effort after three years at Stony Brook, but Barton Childs of Johns Hopkins University has bravely proposed a transition from Oslerian to Garrodian thinking in his splendidly original book *Genetic Medicine: A Logic of Disease* (6).

It was not just a disappointment with medicine that caused me to leave New York, but rather a new kind of opportunity in Texas. Lee Clark, the founding President of the MD Anderson Cancer Center, invited me to start a Cancer Genetics Center in Houston, an opportunity that immediately crystallized my thinking. I had good friends there too, including Margery and Charles (Hez) Shaw. The details became complicated by the decision to start a University of Texas Medical School, and I became the dean of the Graduate School of Biomedical Sciences, where I could appoint state-supported faculty members. We started a Medical Genetics Center with Margery as director, and a Population Genetics Center with Schull as director, with great help from Reuel Stallones, the dean of the School of Public Health. Meanwhile, I published my paper on two mutations to produce retinoblastoma (24) and Margery published a fine paper on chromosomal banding. My paper was communicated to *Proceedings of the National Academy of Sciences of the United State of America* by James (Jim) Neel at the University of Michigan, who had published a fine paper on the genetics of retinoblastoma just before we persuaded Schull to leave there. Jim was understandably unhappy, but he had no retort to my comment that it was his fault that he had such an accomplished colleague.

Houston gave me the opportunity of a fresh approach to the problem of genetics and cancer. Dominantly heritable cancers of several types were known and seemed to promise real "cancer genes." It was clear that inheriting one of these genes was not sufficient for tumor formation. There was a problem with the mechanism of penetrance. Muller had proposed in 1951 that radiation-induced cancer might depend on a series of somatic mutations in addition to an induced one, thus accounting for the long interval between exposure and the appearance of carcinoma. A similar scenario could apply to hereditary cancer. For many adult tumors there could be a long interval to their appearance. However, some pediatric tumors that I was familiar with could appear in dominantly inherited form. Two such tumors were well known: neurofibroma and retinoblastoma. Both tumors could even be present at birth. It seemed that the number of somatic events must be small, possibly even

just one. That number seemed to work well in a model; in fact, a Poisson number of three such "second events" in different target cells fit well with the numbers of tumors observed in genetically predisposed persons (24). These second events could include new mutation, deletion, chromosome loss and, if the gene were recessive in oncogenesis, somatic recombination (26). The normal allele could be viewed as a suppressor of oncogenesis. The nonhereditary form of retinoblastoma fit well to a "two-hit" expectation, so two hits would be present in both forms of the tumor, the difference being in the timing of the first event. In that case, cloning a hereditary cancer gene would be informative for the more common nonhereditary form of a cancer.

One of the happiest developments of my time in Houston was the visit paid by Louise Strong, newly graduated from medical school, to inquire about working with me in cancer genetics along the lines of the retinoblastoma analysis. She was already attuned to this approach because of her undergraduate degree in mathematics. We made a case for similar genetic mechanisms in Wilms' tumor and neuroblastoma, two other embryonal tumors of children (34, 35). With David Anderson, a pioneer in cancer genetics, we also reviewed the full spectrum of hereditary cancer and suggested that all such disorders could lead to the cloning of cancer genes that are also important in the more common nonhereditary forms of cancer (29).

Two genetic "hits" could not explain the most important category of cancer, the carcinomas. However, in some instances they could explain the origin of benign precursors of those carcinomas, which may yet be shown to play a role in all carcinomas. If this is true, then the two events leading up to, and the events immediately following, the precursor state become focal points for measures of intervention. A generalization that can never be completely correct, but can be used as a guiding principle, is that for two-hit malignancies, treatment is critical, whereas in the carcinomas, prevention along the path of progression may be more feasible. In both situations two-hit lesions capture our attention; for the hereditary carcinomas, interference with penetrance is conceivable.

In the mid-1970s human gene cloning was on the horizon but not yet realized, although the promise of its realization gave importance to the localization of hereditary cancer genes, and especially of the retinoblastoma gene, soon to be referred to as *RB1*. The availability of chromosomal banding techniques in the early 1970s made this possible. My involvement in this process came about by accident. I was invited to be a member of a committee of external reviewers of the childhood cancer research program at the Children's Hospital of Philadelphia, and there met Dr. Anna Meadows, whose main research concerned the late effects observed in survivors of childhood cancer, including those with retinoblastoma. Not long thereafter she observed a child with retinoblastoma who seemed somewhat retarded and asked Dr. Warren Nichols to examine the child's constitutional karyotypes for abnormality, whereupon a deletion was found in chromosome 13. I was pleased to answer a request for collaboration that led to our comparison of this case with a few published cases that pointed to band 13q14 as the site of *RB1*

(31), a localization determined independently by Francke & Kung (9). At about this same time I was invited to become the director of the Institute for Cancer Research at the Fox Chase Cancer Center in Philadelphia, an institution I knew for its research, including the discovery of the Philadelphia chromosome, and through my membership on its external advisory committee. Such a move was attractive to me both scientifically and personally, as Anna Meadows and I decided to marry. A mutual friend of ours congratulated me on my two hits.

Soon thereafter, friends and colleagues elsewhere attacked the retinoblastoma problem. At UCLA, Robert Sparkes, whom I had known at the City of Hope, and William Benedict discovered very close linkage between *RB1* and the *esterase D* gene on chromosome 13 (48). In Toronto, Brenda Gallie, Robert Phillips, and Roseline Godbout, using the EsD polymorphism that Sparkes discovered, found that some tumors that occurred in children heterozygous for this polymorphism were hemizygous for the EsD protein, in keeping with the idea that *RB1* mutations are recessive in oncogenesis (12). Benedict, Sparkes, and colleagues described a person who was heterozygous for loss of EsD and whose tumor had no EsD activity (2). Then Webster Cavenee, working with a group that included Louise Strong, the Toronto group, and Thaddeus Dryja, utilized his newly discovered collection of restriction-fragment-length polymorphisms to apply DNA technology to cancer genetics, demonstrating not only the recessive phenomenon, but also the several kinds of second events in oncogenesis, which included those previously predicted, e.g., local mutation, deletion, chromosomal loss, and somatic recombination (5). Three years later, Stephen Friend, a colleague and friend from his days as a pediatric resident at Children's Hospital in Philadelphia, Dryja, Robert Weinberg, and their colleagues isolated the *RB1* gene, the first tumor suppressor gene to be cloned (10); for the first time the mechanism for the penetrance of one hereditary cancer gene was established.

There followed a series of experiments that demonstrated that the Rb protein encoded by *RB1* is a key regulator of the cell cycle in all cells. It was also discovered that this protein, as well as the p53 protein, can be inactivated by proteins produced by DNA tumor viruses, thus effecting both an increase in cell birth rate and a decrease in cell death rate. This startling development followed one in which it was discovered that transforming genes of RNA tumor viruses have normal cellular counterparts in protooncogenes. In a relatively brief period the viral and somatic mutational theories of cancer merged as common mechanisms were identified.

BEYOND TWO HITS: GENETIC CHAOS

In 1983 I left administration and, while still at the Fox Chase Cancer Center, began studying the only animal model for dominantly inherited cancer that I could find, the Eker rat. In 1961 Reidar Eker of Norway described the dominantly inherited predisposition to renal carcinoma in *Rattus norvegicus* (7). He reported that homozygous animals could not be recovered from appropriate matings, and

supposed that such animals died as fetuses. Dr. Eker kindly supplied me with these animals and we began a search for the gene, a search that ended in 1994 with the discovery by my colleague Raymond Yeung that the cause was a mutant of the tuberous sclerosis type 2, or *TSC2*, gene (53). This finding was confirmed by my Japanese colleague, Okio Hino, and his group (37). We also showed a linear dose response for irradiation-induced tumors, suggesting that the tumors were two-hit lesions (15). These tumors were typically adenomas, and we presumed that other genetic changes are responsible for progression to carcinomas. We also discovered that the homozygous state was generally lethal at 10–14 days of fetal life. Another colleague, Cheryl Walker, demonstrated severe abnormality of the brain as a frequent occurrence (44). Mutation of the *TSC2* gene could be considered a recessive, lethal, developmental gene. This phenomenon of recessive fetal lethality has been observed for numerous knockout mice with mutations in dominantly inherited cancer genes, including the *RB1* gene, the first tumor suppressor gene for which the phenomenon was described. This has been a confirmation of the view long held by some investigators that development and cancer are intimately related.

Tuberous sclerosis is one of a group of hereditary diseases called the phakomatoses and unified by the fact that in each condition there are scattered benign lesions, sometimes hamartomas and sometimes adenomas, that are precursors, at a low rate, of malignant tumors. At present, 10 or so disorders are included: Neurofibromatosis (NF) type 1 (*NF1* gene), NF2 (*NF2*), Tuberous Sclerosis 1 (*TSC1*), TSC2 (*TSC2*), von Hippel-Lindau disease (*VHL*), Gorlin's disease or Nevoid Basal Cell Carcinoma syndrome (Patched gene, *PTC*), Cowden disease (*PTEN* gene), Familial Adenomatous Polyposis (*APC* gene), Juvenile Polyposis (*STKII* gene), and Peutz-Jeghers syndrome (*SMAD4* gene) (51). The genes are tumor suppressors whose proteins are active in signal transduction from the cytoplasm to the nucleus. Heterozygotes for mutation of one of these genes develop the benign lesions as a result of a somatic mutation in the second allele. Hundreds of such lesions may occur in the target tissue, some of which, after many years, typically become malignant. These two hits are insufficient for invasion and metastasis, and other genetic events are necessary. It may be that all carcinomas arise from benign precursor lesions that are produced by two hits, and that the two-hit lesions may arise in either genetically predisposed, following one somatic mutation, or normal individuals following two somatic mutations. Such a phenomenon presents an opportunity to intervene on the path to cancer, either in preventing a second hit, or in preventing or delaying subsequent events; i.e., there could be interference with genetic penetrance.

Hereditary retinoblastoma and the phakomatoses have incidences in large populations of two to five per 10^5 births. For each there is a significant fraction of cases, in the approximate range of 20% to 80%, that represent new germline mutations. For retinoblastoma this number is 80%, for NF1 50%, and for FAP approximately 20%. Thus, the incidences have been determined by mutational equilibrium, where the gene frequency (q) is determined by mutation rate (μ) and a coefficient of

selection (s), according to the relationship $\mu = sq$. Mutation rates are typically in the range of 0.5–2.0 per 10^5 per locus per generation, although NF1 is unusual, with a gene mutation rate of approximately 8 per 10^5 births, one of the highest rates known for a human disease.

There has been no evidence of any inherited susceptibility to cancer having incidences determined by heterozygous advantage, but a few examples have frequencies that are too high to be explained by high mutation rates. The most famous of these involves the genes *BRCA1* and *BRCA2*, both of which predispose especially to breast cancer. Incidences in numerous populations are greater than 1 per 1000 births and in a few are of the order of 1%. New mutants are very rare, so high mutation rate is not an explanation. Some families seem to carry a mutation that is hundreds of years old. The best explanation seems to be that in past generations very few women died from breast cancer before the end of the reproductive period, so the coefficient of selection was very low, perhaps of the order of magnitude of 1%. My conclusion is that hereditary predisposition to cancer has generally contributed very little to the genetic load in humans because the heritable forms that cause death before the end of the reproductive period are typically uncommon (27). This situation is evidently changing now because breast cancer has been occurring earlier over the past century or so and is now the chief cause of death in the United States among women 35–50 years of age, and the average age of mothers at childbirth is increasing.

There is still much to be learned about the penetrance of hereditary cancer genes because the common carcinomas for which there may be genetic predisposition arise many years after birth, as with colon carcinoma. In Familial Adenomatous Polyposis benign polyps arise in childhood, but carcinomas typically occur in adults and are infrequent events among hundreds of polyps. There is much evidence that other genetic changes occur, as well as abundant evidence that the carcinomas are genetically unstable, with abnormalities of both number and structure of chromosomes. Now the main question to answer is whether the instability itself is responsible for progression or whether it is some accompanying aberrations in specific genes that are responsible. Efforts to answer this question have revealed important roles for genes that are responsible for repairing endogenous DNA damage, including double-strand breaks in DNA. The latter occur at a rate of approximately 50 per cell division (52), so any failure of repair will cause cell death or, in the absence of a normal apoptotic response, survival with genomic instability. The result is a caricature of normal growth and development, a kind of cellular bioterrorism. Surprisingly, two genes that are important in the repair of double-strand breaks are *BRCA1* and *BRCA2*. Heterozygous carriers of mutation in either of these two genes acquire somatic cells that have undergone mutation or loss of the second allele, thereby rendering the cell defective in DNA repair at other sites, with resultant aneuploidy, structural aberrations, and mutations.

Careers can never be completely planned, but a retrospective view after 60 or so years may reveal more pattern than realized. My interests began in physical science and mathematics, suddenly changing to genetics and developmental biology, then

to medicine (pediatrics), then shifting from congenital defects and inborn errors of metabolism to common diseases of adults, notably cancer. The discovery that the genetic material is DNA is followed after all of these years with a concern for the fidelity of its replication over time and the chaos that is cancer when that fidelity fails. It has been an exciting 60 years.

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