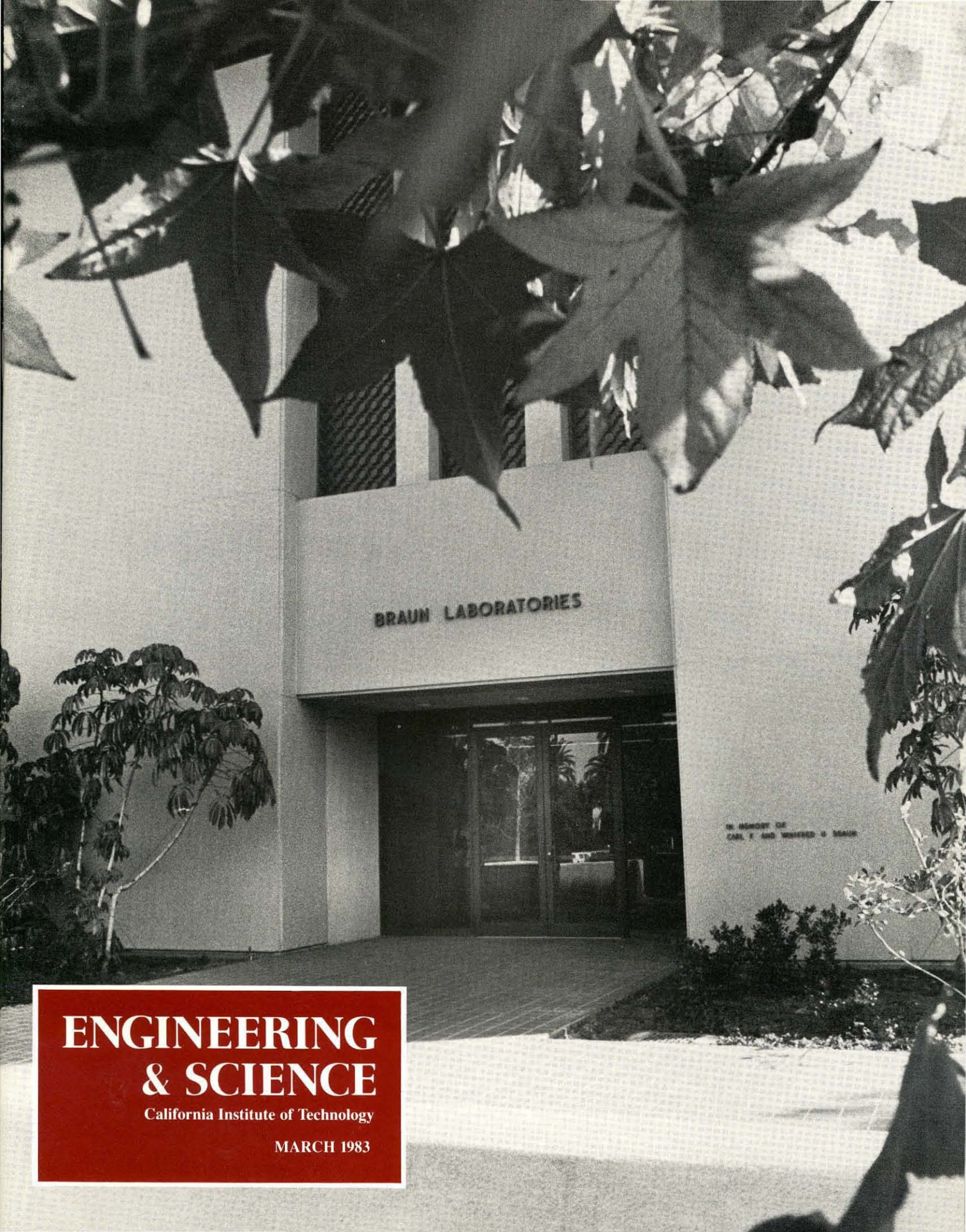
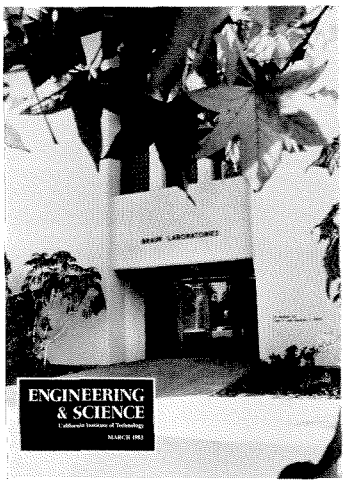


ENGINEERING & SCIENCE

California Institute of Technology

MARCH 1983

In This Issue



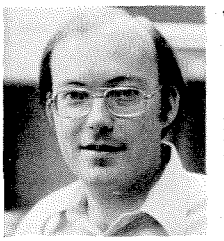
Braun Labs

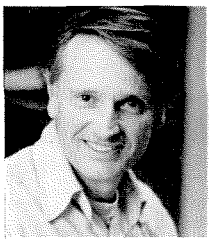
On the cover — the west entrance to the new Braun Laboratories in Memory of Carl F and Winifred H Braun. These doors, of course, represent the entry to more than a building; they are also a gateway to an expanded level of basic research in molecular biology, immunology, and cell-surface chemistry at Caltech. Beginning on page 4, *E&S* presents a glimpse of some of the work now under way in these fields.

Contributors

In the January issue of *E&S*, we referred to Leroy Hood as "many-titled," which seems fair enough. He is the Bowles Professor of Biology, the chairman of the Division of Biology, and the director of the cancer center in the new Braun Labs. He is also twice a doctor, holding both an MD from Johns Hopkins and a PhD from Caltech. Before joining the Caltech faculty in 1970, Hood was for three years a senior investigator at the National Cancer Institute in Bethesda, Maryland.

Hood's co-author in this issue, Senior Research Fellow Michael Hunkapiller, came to Caltech as a graduate student in 1970 and received his PhD in 1974. He has spent much of the last ten years developing instruments and information in the microchemical instrumentation facility at the Institute. In "Biotechnology and Medicine of the Future," which begins on page 6, he and Hood report on some of this research and its implications for society.



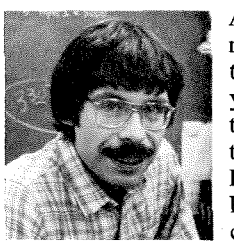


John Richards, professor of organic chemistry, received his BS and PhD degrees at UC Berkeley, sandwiching between them a BSc at Oxford, where he spent two years as a Rhodes Scholar. After two years as an instructor at Harvard, he came to Caltech in 1957.

Speaking of Richards at the dedication of the Braun Labs, the chairman of his division, Harry Gray, said: "Jack Richards has a well-deserved international reputation for his research in oxygen transport in red blood cells, in enzyme catalysis, and in molecular immunology. What the Richards group is learning about abnormal hemoglobins may soon play a very important role in control of blood-cell diseases such as sickle cell anemia. In enzyme catalysis, the group is using the most modern methods of molecular biology to tailor the structures of enzymes so as to find out exactly how they work. Finally, his work in molecular immunology has as a central theme the interaction of antibodies with antigens." In "Structure and Function in Biochemistry," which begins on page 14, Richards describes some of this work.

Although Ellen Rothenberg's laboratories will be located in Church Lab, her work in cellular immunology is very much a part of the new direction in biology that Braun Labs represent. In "How to Find Needles in Haystacks: Fluorescence-Activated Cell Sorting" beginning on page 20, she describes the new instrument used to isolate cell subpopulations and determine correlated cell properties.

Rothenberg received her AB from Harvard and her PhD from MIT in 1977. Before coming to Caltech last June as an assistant professor, she worked at MIT's Center for Cancer Research, the Memorial Sloan-Kettering Cancer Center, and the Salk Institute for Biological Studies.



Among the new generation of young scientists attracted to the Braun Labs is Carl Parker, who came to Caltech in 1981 as assistant professor of chemical biology. He received his BA from the University of Rochester and PhD from Washington University (1977). His research on the mechanisms regulating structural gene transcription in *Drosophila melanogaster* was begun as a postdoctoral fellow at Stanford's School of Medicine.

Parker's article, "Biochemical Studies on Gene Expression in Higher Organisms" beginning on page 18, describes his work on gene transcription using Drosophila's heat-shock genes as a model system.

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ENGINEERING & SCIENCE

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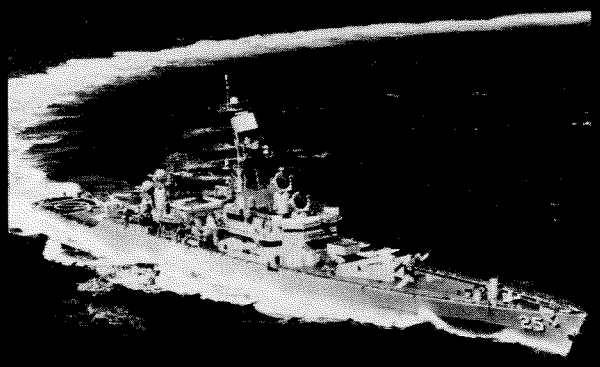

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Chemistry and Biology in the Braun Laboratories

An Introduction by Leroy Hood

THE BRAUN LABORATORIES and their commitment to immunology and molecular biology arose from a consideration in the early 1970s about appropriate future directions in biology. These deliberations led to a program, the Medical Sciences Program, which Caltech's president, Harold Brown, approved in 1975. This program had three major components. First, the direction was immunology and molecular biology, two areas that were having tremendous impact on both fundamental and applied medical problems. Second, three new professorial appointments were to be made in these areas. The emphasis was to be on selecting interdisciplinary scientists, excellent in fundamental research, but with a real commitment to the application of their research efforts. Finally, the Medical Sciences Program was to be housed in a new structure now known as the Braun Laboratories in Memory of Carl F and Winifred H Braun.

Caltech has made fundamental contributions to immunology and to the genetics that led to molecular biology for the past 50 years. The Division of Biology was founded in 1928, when Thomas Hunt Morgan moved from Columbia to carry on his classic work with the genetics of fruit flies, for which he received the Nobel Prize in

1933. Classical genetics led quite naturally to biochemical genetics, which was formulated and exploited by George Beadle in the 1930s and 1940s. Beadle received the Nobel Prize for this work in 1958. Max Delbrück worked on bacteriophage genetics in the 1940s, the 1950s, and the 1960s. These efforts, for which he received the Nobel Prize in 1969, laid the foundations of modern molecular genetics. In the 1970s, the progression of these diverse lines of genetic research had placed Caltech in an ideal position to participate in development of the new genetic engineering or recombinant DNA techniques. In the past ten years, scientists at Caltech have made substantial contributions to the development and application of recombinant DNA research.

In the past ten years Caltech also has developed a unique microchemical facility containing machines that allow scientists to analyze and synthesize genes and proteins in ways that were heretofore impossible. The development of this facility has in large part been made possible by the unique attributes of Caltech — excellent machine and electronics shops, a close relationship with the superb engineers of the Jet Propulsion Laboratory, and a casual and intimate atmosphere that has facilitated the interactions of biologists, chemists, and engineers. Approaches made possible by the microchemical facility and recombinant DNA techniques have proved remarkably synergistic in attacking fundamental problems of developmental biology.

Caltech's contributions to immunology have been almost as striking. Linus Pauling and Dan Campbell started in the 1940s to study the antibody molecule and determine how it carried out its function of protecting man against viral and bacterial infections. In the mid-1940s Ray Owen moved from Wisconsin and began to study the fascinating question of what prevents the immune system from attacking the individual's own proteins and cells. The question of immune tolerance to self is one of the fundamentally important areas in learning how to avoid the rejection of organ transplants.

In the 1960s, when I was a graduate student here with Bill Dreyer, he and a postdoctoral fellow, Claude Bennett, formulated a hypothesis which revolutionized immunology. This then radical concept, which suggested that antibody genes could jump around on chromosomes, has turned out to be a cornerstone of modern molecular immunology. Against this background, some striking advances in the understanding of immunologically relevant genes have occurred here in the past ten years, and Caltech has come to be known as a center of molecular immunology.

Caltech is currently one of the nationally ranked departments in molecular biology and will become even better with the recent acquisition of two outstanding senior molecular biologists from San Diego, John Abelson and Mel Simon, both of whom will move into the Braun Laboratories in 1983. A first-rate young immunologist, Ellen Rothenberg, also has recently moved here from the Salk Institute in La Jolla. These scientists, as well as the outstanding immunologists and molecular biologists already here at Caltech, are interested in a variety of fundamental problems. How do genes, such as those for antibodies and the transplantation antigens that cause the rejection of grafted organs, function? Can a precise molecular mapping of human chromosomes be determined? Can techniques for protein engineering be developed, that is, can genes be altered in test tubes so that the proteins they encode will have new and useful properties? Novel and innovative instruments continue to be developed in the microchemical facility.

As Caltech looks to the 1980s and 1990s, we would like to move in several new directions — plant molecular biology and molecular developmental neurobiology — and at the same time maintain and extend our strengths in molecular and cellular biology and neurobiology. The opportunities for future research appear practically unlimited. It will be possible to isolate biomedically interesting human genes rapidly. These genes can be placed in bacteria, yeast, or human cells to synthesize large quantities of their respective proteins for diagnostic or therapeutic purposes. Antibody molecules will be fashioned with almost any desired specificity. We will come to understand how cancer genes function and use this knowledge to design rational diagnostic and therapeutic approaches for tumors. We will be able to synthesize complete human genes. We will be able to synthesize small fragments of proteins that can be used to generate vaccines that are difficult to produce now for a variety of technical reasons. Thus the Braun Laboratories will not only represent a point of enormous excitement now, but in many ways they will serve as a focus for the development of new biotechnologies and new avenues for scientific exploration in the future. The future is indeed bright, and Caltech and the Braun Laboratories perhaps occupy a unique position in being able to take advantage of these opportunities. For all of this, we must thank John Braun, his family, and all the rest who made the Braun Laboratories possible. This issue of *Engineering & Science* presents, in the first four articles, a discussion of some of the work in progress. □

Biotechnology and Medicine of the Future

by Leroy Hood and Michael Hunkapiller

The 1980s appear to be the decade of biotechnology, and two of its aspects — recombinant DNA and microchemical instrumentation — will impinge greatly on medicine of the future.

THE 1980s APPEAR to be the decade of biotechnology. The striking new advances in biotechnology appear to offer virtually unlimited opportunities for attacking fundamental and applied problems in medicine, agriculture, energy, and chemical synthesis. We will discuss two aspects of biotechnology, recombinant DNA and microchemical instrumentation, and will consider how these advances impinge on medicine of the future.

DNA, Genes, and Development

The human organism starts its development as a single cell, the fertilized egg. This cell goes through a highly programmed and reproducible series of developmental stages. Initially the single cell doubles again and again to generate a ball of cells; then further division and cell migration lead to a hollow sphere, which elongates into a cylinder. The cylinder flattens and folds into three layers which in turn eventually give rise to the three fundamental cellular types from which the tissues and organs of the human organism are fashioned. Thus, from a single cell a human composed of 10^{14} cells with hundreds of specialized types develops. Two aspects of this developmental process raise intriguing questions that can be approached with the techniques of recombinant DNA. How do specialized cells turn on the particular genes they need for their differentiated functions? For example, how can red blood cells turn on the gene for making hemoglobin, the oxy-

gen-carrying molecule of human organisms? Second, what is the nature of the developmental program which directs every human organism to differentiate and mature in precisely the same way?

The information for expressing specialized proteins, such as hemoglobins, or controlling precisely the developmental pathway of the human organism is contained, in part, in the DNA language of its chromosomes. We can think of each human cell as having a library of encyclopedias with instructions on how to produce humans. The DNA language is made up of letters and words, just as the English language is. The DNA encyclopedias of each human cell contain 3 billion different letters of the DNA language. In DNA language the average word contains approximately 1000 letters. Hence human cells potentially have the information for 3 million different DNA words. Recombinant DNA technology permits scientists to isolate a single DNA word, place it in a test tube, and then study how this word contributes to the development of human organisms.

The 23 chromosomes contained in every human cell are the blueprint repositories containing the information for constructing human beings, and individual units of information or the words of the DNA language contained within the chromosomes are called genes (Figure 1). The gene or word is translated into the functional entities, called proteins, by a complex, computer-precise process denoted protein synthesis. The information contained in each gene is read from the so-called master computer tape, the genes of the human chromosomes, into a working computer tape called messenger RNA, which ultimately can be translated in special cellular machines into the proteins that give our body shape, form, and function (Figure 1).

The elegantly simple DNA molecule that makes up the informational backbone of the chromosomes has been beautifully fashioned for its primary function of information storage and transfer. A DNA molecule is made up of two oppositely oriented chains of nucleic acids, which are composed of four different letters or nucleotides — A, T, G, and C. (Figure 2). The two chains of the DNA molecule exhibit a molecular complementarity that confers upon DNA all of its fundamental properties. The letter A in one strand always pairs with the letter T in the second strand, as does the letter G always pair with the letter C (Figure 2). This letter pairing means that one DNA strand in a DNA molecule is the molecular complement of its partner, and, if separated, the two strands can readily find one another even in a complex mixture of DNA strands and

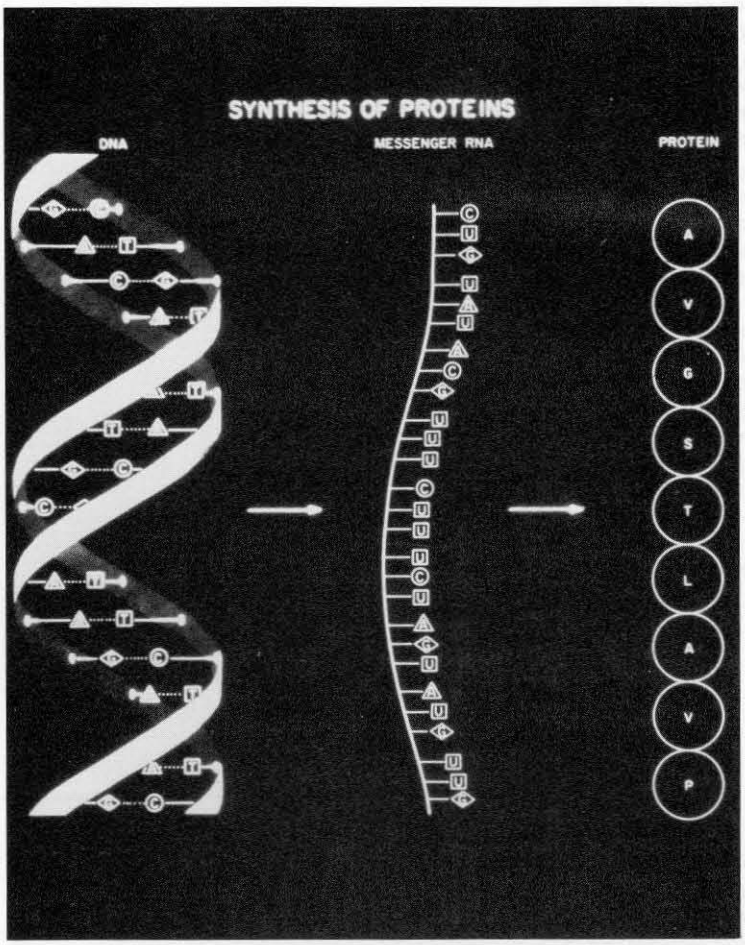
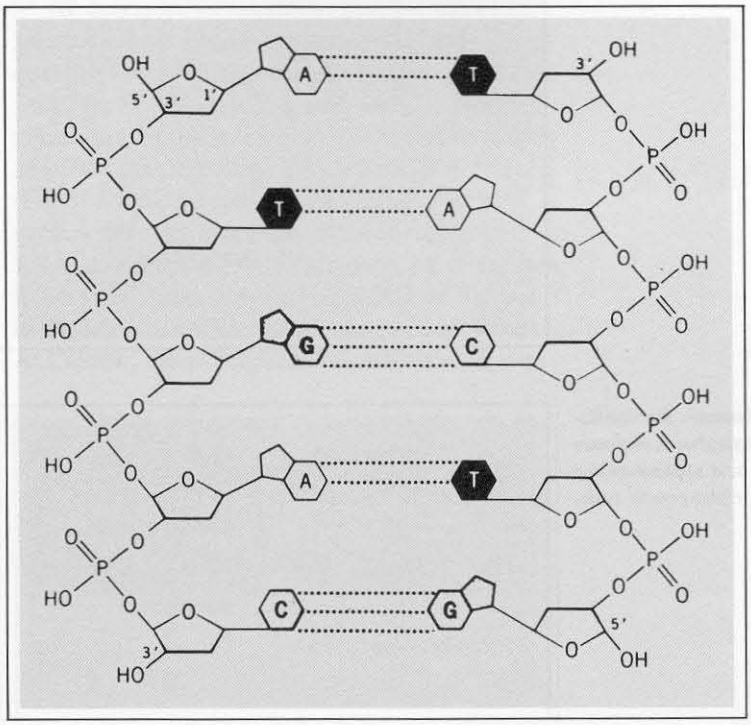


Figure 1 (above) illustrates the molecular relationship between DNA, RNA, and protein. Figure 2 (below) is a depiction of the Structure of DNA emphasizing the complementary base pairing between the two strands.



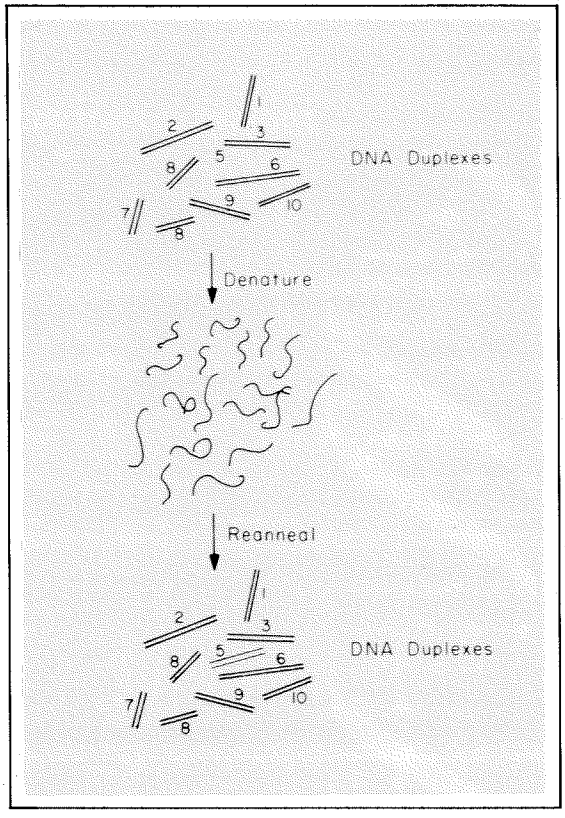


Figure 3. The denatured, separated strands of DNA molecules base pair specifically to their complements and reform the double-stranded structure when the denaturing conditions are removed.

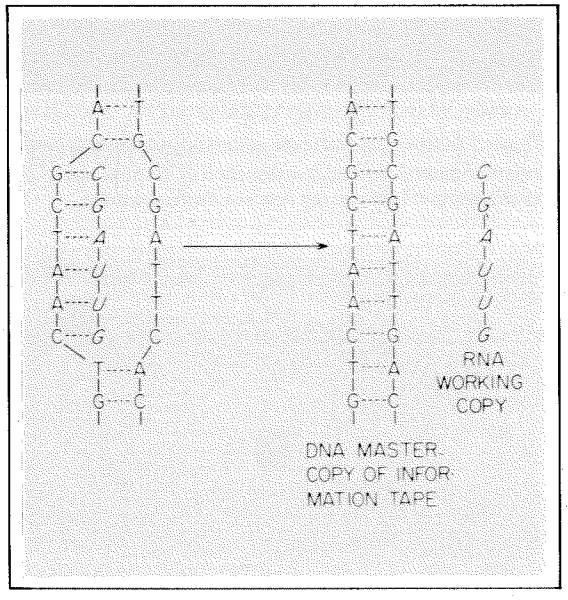


Figure 4 shows the transcription of DNA into RNA, in which the molecule of RNA is formed with a base sequence that is complementary to one of the two original DNA strands.

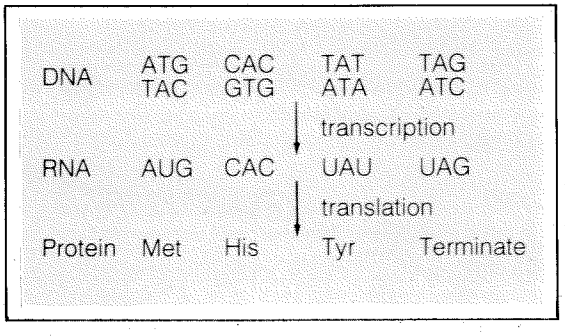


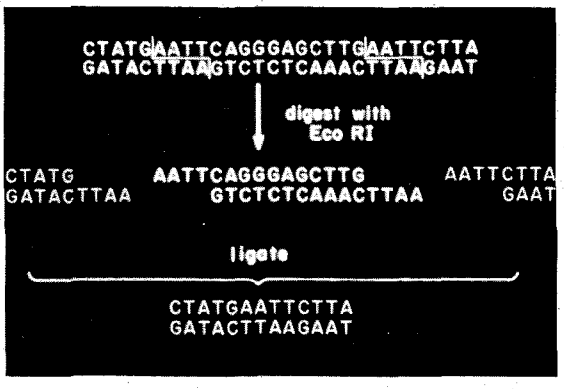
Figure 5 illustrates the translation of nucleic acid sequence into amino acid sequence using the triplet genetic code.

reassociate to form the duplex DNA molecule (Figure 3). The implications of this molecular complementarity give us fundamental insight into how DNA carries out its two most important functions — the replication of itself and protein synthesis. In order to replicate and create the DNA molecules for new cells, one DNA strand can serve as a template against which the second DNA strand is constructed by assembling together the letters in the new chain via the rules of molecular complementarity. Thus the two strands of a DNA molecule can separate and reassemble their complementary partners to generate two DNA molecules from one. Likewise, information from that functional unit of the DNA molecules, the gene, is transferred to the RNA computer tape, again by the process of molecular complementarity in which the RNA molecule is synthesized using one DNA strand as a template (Figure 4). This is possible because the RNA language also contains four different letters each of which uniquely pairs with a single DNA letter. The fundamental basis for recombinant DNA techniques resides in molecular complementarity because separated DNA chains can find and interact with their complementary partners.

The molecular code by which information is transferred from the DNA master computer tape through the RNA working computer tape and ultimately to proteins is a fascinating process (Figure 5). The DNA molecule is double stranded and its information is stored via a language containing four letters. The RNA molecule is single stranded and also uses, as noted above, a language with four letters. Proteins, the functional units expressing gene information, are linear chains composed of 20 different letters called amino acids. Each amino acid is specified by a trio of letters in DNA and RNA language. Thus, triplets of DNA letters can be translated into single protein letters, and, conversely, protein letters can be reverse translated into DNA triplets. This ability to translate the genetic code dictionary in both the forward and reverse directions is fundamental to the use of the microchemical instrumentation we shall describe later.

Recombinant DNA Techniques

The breakthrough in the development of recombinant DNA techniques came with the identification of enzymes that could cleave DNA molecules at precise points (restriction endonucleases) and enzymes that permit the joining together of distinct DNA fragments (ligases). The typical human chromosome contains approximately 10^8 nucleotide pairs. Restriction enzymes may cleave this large DNA molecule into regular gene-sized



fragments (Figure 6). The restriction endonucleases cleave DNA molecules at precise points determined by nucleotide recognition sites of four or six letters. For example, one restriction endonuclease cleaves all DNA molecules each time the base sequence GAATTC appears. Often the DNA strands are cleaved in an offset manner so that staggered single-stranded ends remain that exhibit a molecular complementarity for one another that allows any fragments produced by a particular restriction enzyme to be joined back together (Figure 6). Restriction endonucleases arose in bacteria as a defensive mechanism for destroying foreign DNAs. The bacterial DNAs are protected from cleavage by modifying nucleotides in the restriction enzyme recognition signal so that their own particular restriction enzymes cannot cleave their own DNA. This fortunate accident of nature has given molecular biologists hundreds of different restriction endonucleases to facilitate the cloning of human genes.

A second category of enzymes called ligases permits DNA fragments to be joined together, often using the molecular complementarity of their staggered ends (Figure 6). The ability to join together any two fragments of DNA permits human gene fragments to be spliced into small DNA circles called plasmids that can be grown up in large quantities in bacteria such as *E. coli* (Figure 7). The approach is to genetically modify the plasmid so that a particular restriction enzyme cleaves it at a single site. Then fragments of human genes cleaved by the same restriction enzyme can be inserted into plasmids, the human and plasmid DNAs can be ligated together, and the recombinant plasmids can be used to infect the bacteria *E. coli*. The recombinant plasmid is amplified to make hundreds of copies of the human gene in each bacterium (Figure 7). Thus large quantities of the particular human gene can be grown up in bacteria. The individual *E. coli* containing plasmids with different human genes can then be plated out on appropriate media to generate thousands of individual colonies, which

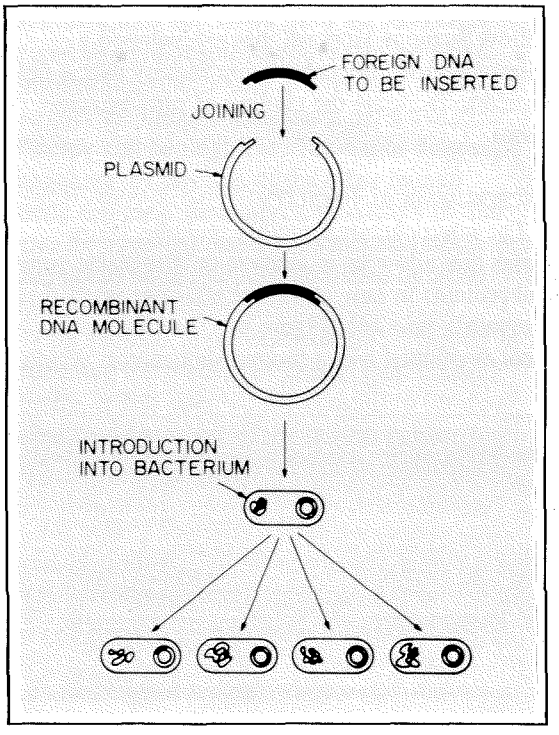


Figure 6 at the far left depicts the action of restriction enzymes and ligases. The restriction enzyme recognizes specific base sequences and clips the DNA strands at these points, leaving ends of uneven length on the original complementary strands. The DNA fragments can then base pair at these ends with any piece of DNA cut by the same enzyme, and hence possessing the appropriate complementary base sequences. The splicing action of the ligases can then be used to form an entirely new hybrid DNA sequence from the two fragments. Figure 7 (near left) is a schematic representation of the cloning of human gene fragments into plasmids and the plasmids into E. coli.

in turn can be screened to identify the bacterial colony containing the particular human gene of interest.

Rare-Message Genes

In principle the cloning procedure described above will lead to 3 million different clones of *E. coli*, each containing a different human gene. The critical question is how, from among the 3 million bacteria with their human genes, the single bacterium that contains the gene of interest — for example, an interferon gene — can be selected. If the gene in question synthesizes large quantities of the messenger RNA, such as do the genes for hemoglobin and antibodies, molecular probes can be made from the RNAs which readily allow the corresponding genes to be cloned (Figure 8). Conversely, if the gene of interest synthesizes very small quantities of messenger RNA, as is the case with the interferon gene, then it is termed a rare-message gene (Figure 8), and its isolation is a difficult and complicated process. At Caltech, the two authors in collaboration with

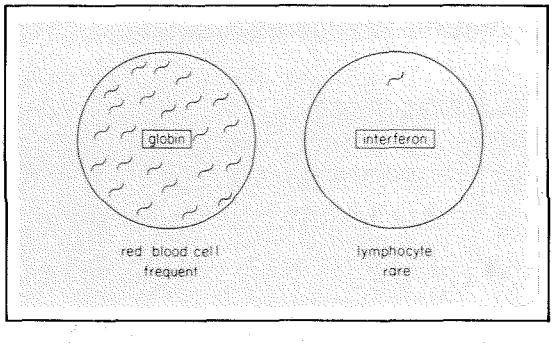


Figure 8. Examples of rarely and frequently expressed messenger RNAs.

Professor of Biology William Dreyer and Staff Fellow Suzanna Horvath, have developed a microchemical instrumentation facility that is designed to facilitate the cloning of rare-message genes.

MICROCHEMICAL FACILITY

The microchemical facility includes instruments that allow us to analyze or synthesize genes and proteins in new and more effective ways than previously possible (Figure 9). The first instrument developed was a protein sequenator, which determines the linear order of amino acids in a protein by acting as a pair of molecular scissors to remove amino acid subunits from the proteins one at a time. A second analytic instrument must be used to identify each amino acid. The main virtue of the Caltech microsequenator is that it is approximately 100 times more sensitive than other sequenators. Indeed, the Caltech sequenator is so effective that it could be ten times more sensitive if we had an analytic instrument of sufficient sensitivity to characterize the individual amino acids at these low levels.

To circumvent this difficulty, we have, in collaboration with Professor Dreyer, spent the last six years collaborating with engineers at the Jet Propulsion Laboratory to design an ultrasensitive mass spectrometer that is capable of analyzing amino acid residues at levels far below those previously possible. This instrument is nearing completion and is sufficiently sensitive that we can use the highest resolution analytic separation tool in protein biochemistry, two-dimensional gel electrophoresis, as a tool for preparing sufficient quantities of protein for sequence analysis with the microsequenator (Figure 10).

The importance of obtaining this very high level of sensitivity for the analysis of proteins can be understood in the context of the third instrument, whose development we have just finished. The DNA synthesizer, or so-called gene machine, has the capacity to synthesize fragments of genes relatively rapidly. It is interesting to note that although the chemistries for protein-sequence analysis and DNA synthesis are very different, the instrumentation and programming requirements for these two chemistries are remarkably similar, and indeed, these two machines — the protein sequenator and DNA synthesizer — are strikingly similar to one another.

These three machines permit us to approach the routine and rapid cloning of rare-message genes in the following manner (Figure 11). Often small amounts of a rare-message gene product such as human interferon can readily be obtained for protein sequence analysis. After a portion of the protein has been analyzed by the protein sequenator, this sequence is reverse translated into DNA language. A small DNA fragment 12-17 nucleotides in length is then synthesized with the DNA synthesizer and used as a molecular probe to clone the rare-message gene. Thus, a task that took in the past two or even three years to achieve, in the future can be accomplished in less than one month. This use of the microchemical facility for the cloning of rare-message genes accordingly opens up the potential for the cloning of an enormous variety of interesting and biomedically rele-

Figure 9. *A capsule description of the instruments that comprise the Caltech microchemical facility and the primary function of each.*

1. Protein Microsequenator	– determines order of amino acids in proteins
2. Peptide Synthesizer	– synthesizes small peptides and fragments of proteins
3. DNA Synthesizer	– synthesizes oligonucleotides (genes)
4. DNA Sequenator	– determines order of nucleotides in genes
5. Gene Analyzer	– fingerprints genes

Figure 10 *is a diagram of the separation of complex protein mixtures by two-dimensional polyacrylamide gel electrophoresis. A: The proteins are separated in the first dimension on the basis of their molecular size. B: They are then separated in a second dimension on the basis of electronic charge on their surface. This technique allows separation of several thousand proteins in a single experiment.*

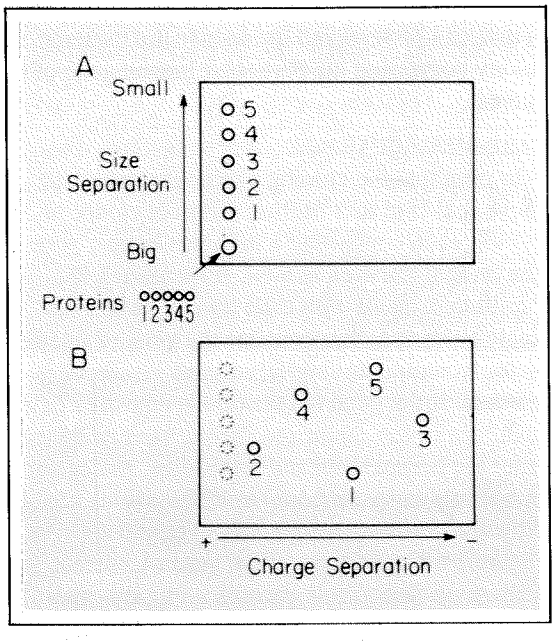
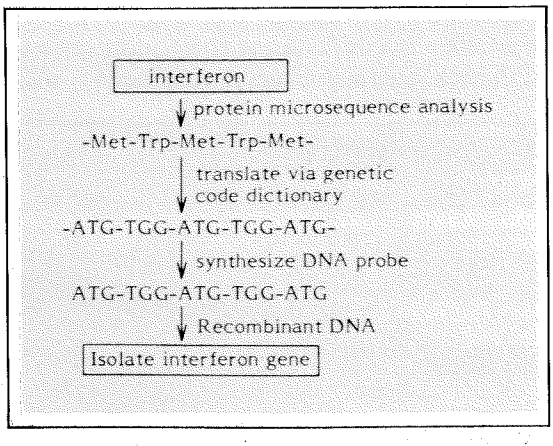


Figure 11 *gives a strategy for cloning of rare-message genes based on synthesis of nucleic acid probes using protein structure obtained by microsequencing.*



vant human genes. Indeed, we hope to be able to clone in the future virtually any gene whose protein product can be visualized on a two-dimensional gel (Figure 10). Thus it will be possible to clone thousands of human genes that were heretofore inaccessible to the techniques of recombinant DNA.

We also are at work designing a machine that we hope will eventually allow us to fingerprint human genes. If we are successful in this endeavor, gene fingerprinting will open up some very exciting new areas for human medical genetics. These two interrelated biotechnologies — recombinant DNA and microchemical instrumentation — will have a great impact on medicine of the future.

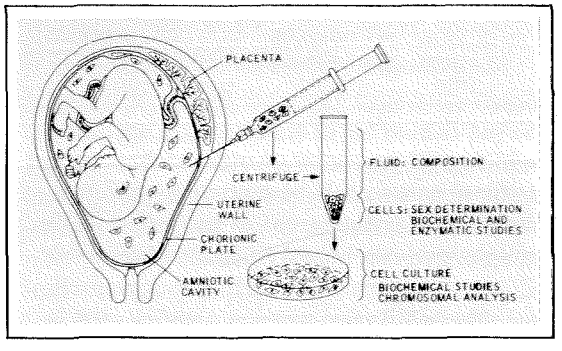


Figure 12 is a schematic representation of analysis of fetal chromosomes by amniocentesis.

Human Genetic Diseases

Approximately 2500 different human genetic diseases have been described. Some of these can be diagnosed prenatally by a procedure called amniocentesis (Figure 12). In this procedure a needle is placed in the amniotic sac of the unborn fetus and used to withdraw fluid and cells that are derived primarily from the lungs of the fetus. The fluid and cells can be examined to determine whether they have biochemical abnormalities indicative of any one of approximately 200 human genetic diseases. Mongolism, for example, can readily be detected by the presence of an extra human chromosome 21 in the cells of the fetus. Tay-Sachs disease can be readily diagnosed because it has a protein abnormality that is present in the amniotic fluid. The techniques of recombinant DNA, at least in theory, offer us the potential to diagnose directly virtually every one of the 2500 different human genetic diseases. The approach would be to fingerprint human genes so as to distinguish normal and abnormal genes. The fingerprinting would compare the sizes of DNA fragments from the gene of interest produced by restriction enzymes (Figure 13). DNA from normal individuals and DNA from the fetal cells derived by amniocentesis could thus be fingerprinted to unambiguously determine whether, for example, the unborn fetus has a defective gene for sickle cell hemoglobin, Tay-Sachs disease, mongolism, or any one of the other human genetic diseases. Recently, a laboratory in San Francisco reported that the normal and sickle cell genes could be readily distinguished by gene fingerprinting.

If we succeed in the microchemical facility in automating the process of human gene fingerprinting, it will open up the possibility for diagnosing *in utero* many of the human genetic diseases. This type of analysis will be particularly important with diseases such as Huntington's chorea, which do not manifest themselves until the individual is 30 or 40 years old. At this time the afflicted person undergoes rapid deterioration in a particular region of his brain. There is no therapy for these patients. Since this disease can be caused by just a single copy of the defective gene, the tragedy is that the carriers of this gene defect often have children before they manifest the disease in their middle adult life. Their children have a 50 percent probability of acquiring the disease. It is psychologically devastating for those children to know that they have to live 30 or 40 years before learning whether they have this inevitably fatal genetic disease. Thus the fingerprinting of human genes will afford a revolutionary and unique opportunity for diagnosing human genetic disease *in utero* in time for a therapeutic abortion or other appropriate measures.

Cancer Genes

One of the most fundamental advances in cancer biology in the last ten years has been the isolation of the so-called cancer genes using recombinant DNA technology. These are genes that have the ability, when introduced into cells, to convert a normal cell into a cancerous cell

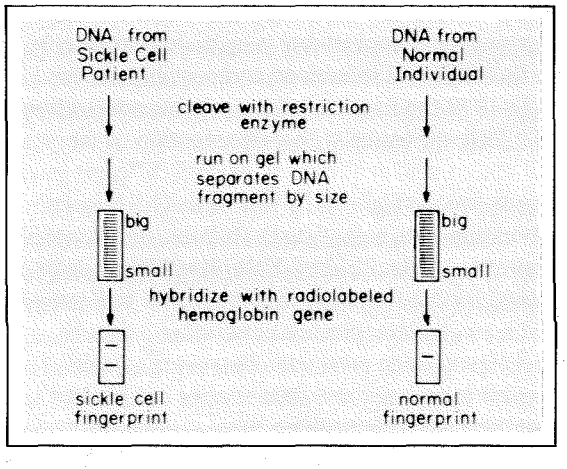


Figure 13 illustrates analysis of human genes by fingerprinting, using one-dimensional polyacrylamide gel electrophoresis and specific DNA probes.

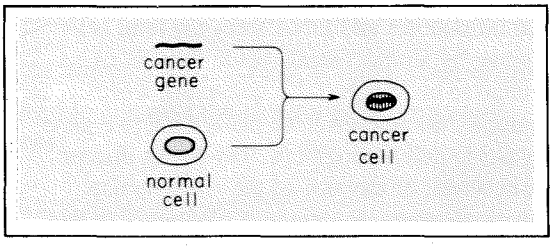


Figure 14. Some types of foreign genes are oncogenic. When they become integrated into a host cell's chromosome, they may help trigger the cancerous state in that cell.

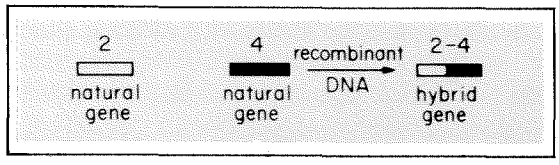
(Figure 14). The isolation of cancer genes presents revolutionary opportunities for understanding the molecular basis for cancer in that these genes can be studied individually to determine how they cause cancer. One of the striking features about cancer genes is that every human has in his chromosomes many normal genes that are remarkably similar to the cancer genes. Indeed, one scientist at MIT has been able to demonstrate that the cancer gene associated with human bladder tumors differs from its normal counterpart by just a single nucleotide in the entire gene. This is a revolutionary observation and raises many fascinating questions about what causes cancer genes to become different from their normal counterparts and what kinds of functions they carry out to actually cause the tumor process. Can environmental agents, for example, convert normal genes into cancer genes? These are questions that the techniques of modern recombinant DNA technology will readily allow us to begin to answer in the future.

Interferon

The cloning of the gene for interferon was a scientific advance that fascinated the general public, whose interest in interferon arose from its potential use as an antitumor agent. The history of interferon is interesting though remarkably brief. Interferon was first identified in 1957, and until recently it was possible to isolate this protein only in extremely small quantities. The entire world's output in 1979 was 0.1 oz., and it cost approximately $20 billion per pound. Even the small amounts of interferon available in the 1970s led to a great deal of clinical excitement because this molecule appeared to play an important role in fighting viral infections and possibly cancer.

In 1979 the first real chemical characterization of interferon began with determination of the partial amino acid sequence of several different interferons here at Caltech by one of us (MH) in collaboration with scientists at DuPont, the National Institutes of Health, and Yale. It was then determined for the first time that interferon was not just a single gene product, but rather a family of different gene products. These amino acid sequence analyses and later studies established that there are at least three basic classes of interferons, denoted alpha interferon, beta interferon, and gamma interferon. In 1980 the alpha and beta interferon genes were cloned. Interestingly, alpha interferon turned out to be encoded by a family of at least 15 different interferon genes. These alpha interferon genes were very similar to one another, and provocative questions were raised as to whether these different genes carried out distinct functions.

The existence of multiple copies of the alpha interferon gene family then permitted molecular biologists to perform a spectacular series of experiments that led to the synthesis of hybrid interferon genes (below). For example, the front half of one interferon gene could be fused to the back half of a second interferon gene to generate a hybrid gene. The interesting observation was that



the biological properties of some of these hybrid interferon genes could be profoundly different from those of either parent. It was reported, for example, that one hybrid alpha interferon gene had enormously enhanced cell-killing properties over either of the two parental proteins used to construct the hybrid. Thus the existence of multigene families for interferons presents the molecular biologist with two kinds of opportunities. First, he can study the normal genes of this family to define their individual functions; perhaps some will be effective antiviral agents and others effective anticancer agents. Second, hybrid genes may be constructed to optimize certain particularly favorable properties in a way that could never have been done in natural genes. The early clinical studies of the various interferon genes suggest that they will indeed be effective agents for dealing with viral infections. Their ability to serve as therapeutic agents for cancer appears less certain.

Replacement Therapy

The techniques of recombinant DNA have already led to the isolation of human genes that encode insulin, growth hormone, interferons, and a variety of other biologically interesting molecules. These genes have been placed in bacteria or yeast in such a manner that large quantities of the corresponding human protein can be synthesized and isolated. Indeed, insulin and interferon derived from these recombinant DNA techniques will soon be commercially available. Thus the recombinant DNA techniques are going to play a

very important role in the isolation of genes that can be used to synthesize products for use in replacement therapy for individuals who have particular genetic deficiencies. As an example, synthetic human insulin will be useful in treating certain diabetics who cannot tolerate the animal insulin given successfully to most diabetics.

Genetic Surgery

With the ability of scientists to clone human genes, the natural question is, Can these genes be transferred directly into human beings who are deficient in these gene functions? For example, can a diabetic patient whose disease arises from a deficiency in the insulin gene be cured by having genetic surgery place good insulin genes in the relevant cells that synthesize insulin? Genetic surgery appears to be a long time in the future. Scientists do not yet know how to position a gene at a precise and appropriate position on the human chromosome. Moreover, they do not know how to place genes in foreign cells such that they can respond appropriately to the normal physiologic signals of their environment. The production of insulin, for example, should be increased when the levels of blood sugar rise. Indeed, with many genetic diseases, the deficiency occurs only in one tissue or population of cells in the organism. It is not clear how to insert functional genes into such a particular cell population. Thus there are a variety of technical difficulties that limit any immediate use of recombinant DNA techniques to carry out genetic engineering on human beings. However, the recent spectacular transfer of the rat growth hormone gene into eight mouse embryos resulted in the production of seven giant mice, presumably generated by the action of the rat growth hormone on the growing mouse embryos. Evidently, it will be possible to genetically engineer humans in the future, but genetic surgery raises interesting social and ethical questions.

Biotechnology and Man

The two biotechnologies that we have discussed here, recombinant DNA and microchemical instrumentation, will not only have a profound impact on the area of biomedical research, but as well on agriculture, energy, the synthesis of carbon-based chemicals, and perhaps even mining. In the long run, the greatest impact of genetic engineering probably will be in agriculture where one will acquire the ability to design plants that fix their own nitrogen, are incredibly resistant to drought and other harsh environmental conditions, are resistant to microorganisms and parasites, and may have enormously enhanced potential for protein and carbohydrate synthesis. Clearly, biotechnology will have a profound impact on modern society that will continue into the indefinite future.

Social and Ethical Questions

The potentialities of these biotechnologies have raised interesting social and ethical questions. What should be the criteria for determining whether to abort an unborn fetus with genetic defects? Clearly most individuals would agree that a fetus with Tay-Sachs disease, a disorder that leads to rapid degeneration of the brain in a newborn child, is a candidate for therapeutic abortion. In contrast, using amniocentesis to determine whether an unborn child is a boy or a girl and then employing a therapeutic abortion to obtain a child with the sex of your choice would be universally condemned. Most genetic diseases or other indications for therapeutic abortions lie between these two extremes, and clearly, society must struggle with these difficult decisions.

Man is now coming to a position where the engineer may engineer himself. Who should decide when and how human engineering can be carried out? What are appropriate experimental models for determining whether the techniques of human engineering are appropriately safe for human beings?

Societal questions are perplexing as well. The interrelationship between the genetic engineering companies and academia poses some serious questions regarding these relationships. On the one hand, the industrial interactions offer potentially enormous resources for academics to carry out their scientific programs. This is not a trivial point in these times when government support for science is waning significantly. At the same time, serious questions must be raised about how much time a professor may spend on outside commercial activities, whether he will employ his laboratory and students in the pursuit of applied research directly for industry, and perhaps most difficult of all, whether he will be forced into a secrecy which is dangerous for the free-exchange style of science that we have known in the past. It is our conviction that the opportunities and dangers can be balanced against one another so that academia and industry each provide for the other something it is missing.

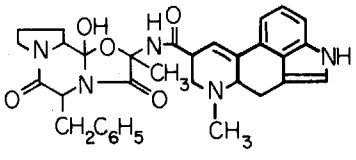
The recombinant DNA techniques and microchemical instrumentation present man with unparalleled opportunities for modifying his environment, even himself. Perhaps the key question that is facing all of us at this juncture is whether man has the wisdom to take advantage of all of these opportunities and use them in an appropriate manner to their fullest potential. □

Structure and Function in Biochemistry

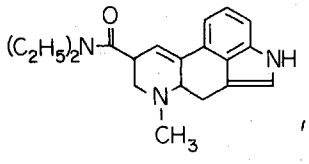
by John H. Richards

STRUCTURE DETERMINES FUNCTION. From the simplest arrangements of a few atoms in a small molecule, such as methyl or ethyl alcohol, to molecules with the exquisite and varied architectures of proteins, the molecular structures mandate uniquely and unambiguously their incredibly diverse properties (as drugs, antibiotics, biological catalysts, hormones, transport agents, cell surface receptors, structural elements such as bones or cartilage, muscles that convert chemical energy into work).

Two examples will show how widely different functions characterize molecules with closely related structures. Methyl alcohol (a toxic agent that can cause blindness) and ethyl alcohol (a mild narcotic that can enliven many a party) differ from one another only by one carbon and two hydrogen atoms. In more complex molecules, ergotamine finds medicinal utility as a vasoconstrictive agent in treating migraine headaches, whereas LSD, which in structural terms represents about one-half the ergotamine skeleton, has protean effects as a mind-bending agent.



Ergotamine



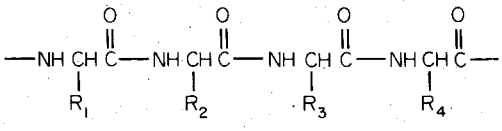
LSD

The molecular structure of LSD is identical to that of those regions of ergotamine emphasized in heavy lines.

Over the last few decades, organic chemists have been wonderfully successful in developing an extensive array of pharmaceutical agents without which modern medicine as we know it would not exist: antibiotics for infectious diseases, anti-inflamatory agents for arthritis, drugs to lower high blood pressure and alleviate congestive heart failure, diuretics to accelerate kidney function, anesthetics, tranquilizers and mood elevators, birth-control pills, and chemotherapeutic agents to combat cancer (some of which have turned the common form of childhood leukemia from a universally fatal disease into one that now has an apparent cure rate of over 60 percent). The possible list is almost endless. In some cases these life-saving drugs have been isolated as natural products from organisms in the world around us — from plants, fungi, bacteria, animals; in many more cases the synthetic art of the organic chemist has proved an essential aspect of the creation of these life-saving or life-enhancing molecules. The indispensable feature that has made all this possible is the present-day ability of the chemist to synthesize almost any organic molecule whose structure holds promise of producing some desirable effect in treating disease.

Until very recently, however, we have lacked the ability to produce specific variations of the molecules that lie at the very heart of every living organism: genes and proteins.

Genes are linear sequences of nucleotides of which there are four types — A (adenine), T (thymine), G (guanine), and C (cytosine). They contain the information that directs the development and functioning of every organism. Information in genes becomes action when translated into proteins. These complex molecules have elaborate and exquisite three-dimensional structures that are determined by the linear sequence of amino acids, of which there are 20 different kinds. This sequence of amino acids, in turn, is determined by the sequences of bases in the genes. Thus one-dimensional information in the form of linear base sequences in the DNA of genes is translated into linear sequences of amino acids in proteins. The basic laws of chemistry and physics then operate on this sequence of amino acids to cause the protein to fold into a precise and beautifully complex three-dimensional structure that posses-



A section of protein. The character of a particular protein is defined by the nature of the side chains (R_1, R_2, R_3, R_4) of each amino acid residue. For this reason subsequent descriptions of changes in protein structures focus on differences in side chains.

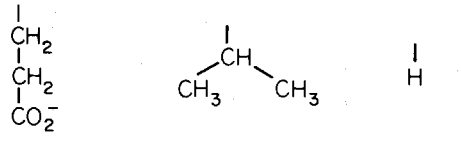
ses a very particular biochemical function. In this way the one-dimensional information of genes is converted into functional, three-dimensional proteins.

For the reasons just outlined, the secret of generating many structural variants of a protein is to be able to alter, in a precisely controlled manner, its sequence of amino acids — and thereby possibly alter its three-dimensional structure and its functional properties. In this way one can approach in a truly rational manner the way the structure of a protein determines its function. By analogy with the contributions of organic chemists in synthesizing many thousands of molecular structures in the spectacularly successful quest for useful agents in medicine and agriculture, we can anticipate that an ability to generate, at will, proteins with any possibly interesting structure will yield an equally rich harvest of useful molecules.

How then can we create proteins with prespecified sequences of amino acids? Today we have procedures that allow us to achieve this objective. This success derives from recent advances in two areas: molecular biology and synthetic organic chemistry. Modern molecular biology has taught us how to introduce essentially any piece of DNA into a microorganism and cause therein the synthesis of the protein that its nucleotide sequence encodes. This is the fundamental advance that has led recently to the production in abundant quantities of such hitherto exceedingly rare but powerful and useful proteins as human insulin, growth hormone, and interferon. Modern synthetic organic chemistry has enabled us to synthesize, rapidly and easily, sequences of nucleotides that constitute pieces of genes. (Indeed, we can synthesize an entire gene, but this represents still a major, very time-consuming endeavor.) The pieces of genes thus synthesized can then be used to alter, in a way precisely specified by the synthetic gene fragment or oligonucleotide, the sequence of bases in the gene for the parent protein — and thereby generate a modified protein with a sequence of amino acids, and therefore a structure and function, never before available.

This ability is tantamount to creating very specific mutants of normal proteins and is formally termed *oligonucleotide directed mutagenesis*. Not only does this lead to proteins with any structure we may specify, but once a single molecule of the gene encoding that protein has been prepared, the protein itself can be produced forever after in microorganisms by the techniques of modern genetic engineering in whatever quantities may be desired.

In nature, mutations occur spontaneously and provide the variations that, together with selective pressures, drive evolution. Even if the mutation is the change in only a single base in a gene with a consequent change of a single amino acid in the protein encoded by that gene, such spontaneous mutations can have profound effects. In the case of the abnormal hemoglobin synthesized by patients with sickle cell anemia, the change is from an A in the codon for glutamic acid to a T in the codon for valine. The mutation of glutamic acid to valine causes the abnormal hemoglobin within red blood cells to gel when it delivers oxygen to the tissues; this gelation in turn causes the deoxygenated cells to sickle, producing the excruciatingly painful crises that characterize this disease. In another example, the change of a single glycine residue to valine in one of the many proteins synthesized by a line of cells grown in tissue culture, causes the cells to become transformed; no longer dividing in a normal controlled manner, they have acquired the characteristics of cancer.



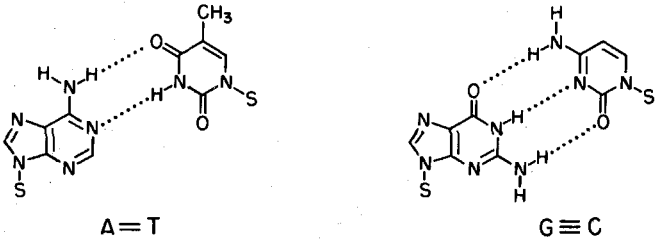
Glutamate Valine Glycine

Glutamate is present at the sixth position in the β-chain of normal adult hemoglobin; valine is present at this position in sickle cell hemoglobin. This is the only change between the two hemoglobins and is the ultimate molecular cause of the ravages of sickle cell anemia. The change from glycine to valine in one of the many proteins produced in a line of cells causes the cell line to become cancerous.

The specific mutations created by oligonucleotide directed mutagenesis involve a combination of organic and biochemical synthesis. Organic synthesis leads to the mutagenic oligonucleotide — generally around 15 nucleotides long — and has become so easy that the task can now be performed by a machine in about 8-10 hours.

The biochemical synthesis is more complex. In the first place, it depends on the necessity for a double-stranded region of DNA as a site at which appropriate enzymes can initiate the replication of DNA, producing thereby a new strand that is the complement of the parent strand. Complementary bases are those pairs that form strongly attractive hydrogen bonds between each other; the recognition of this complementarity of adenine with thy-

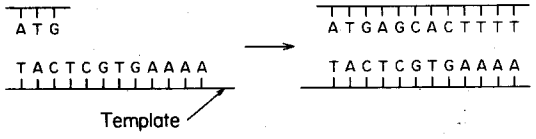
Base Pairing



A=T G≡C

The specificity of Watson-Crick base pairing between A = T and G ≡ C accounts for the preservation of the information content of a gene when it is duplicated.

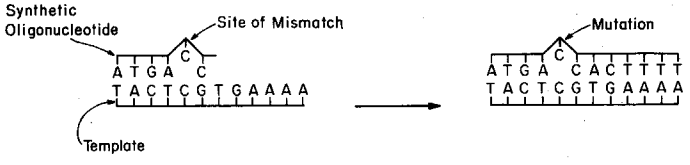
mine and guanine with cytosine played a major role in revealing to Watson and Crick the structure of DNA as a double helix. Thus if one exposes a synthetic oligonucleotide to a long stretch of template DNA that might, for example, constitute a gene, the synthetic oligonucleotide will search along the single-stranded template DNA until it finds a region where its bases complement those of the template, and there the synthetic oligonucleotide will hybridize to form a double-stranded region. The specificity of the complementarity and the rarity of a particular sequence



The biosynthesis of a new strand of DNA uses the information of the template to direct the incorporation of particular nucleotides according to the rules of Watson-Crick base pairing (A-T and G-C).

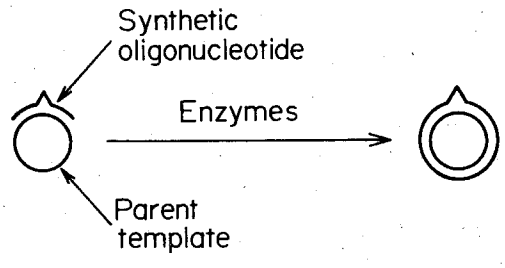
of bases is so high that a synthetic oligonucleotide 15 bases long will find only a single site on a gene many thousands of bases long where all, or most all, of its bases can form Watson-Crick A = T and G = C base pairs. The double-stranded region that results from this hybridization will now serve as an initiation site for enzymes that can complete the synthesis of a strand of new DNA that is complementary to the template.

The secret of introducing a mutation with the synthetic oligonucleotide is that, while most of its bases are indeed complementary to those on the template strand at the site where the oligonucleotide hybridizes, *some are not*. And these will be incorporated into the newly synthesized strand,

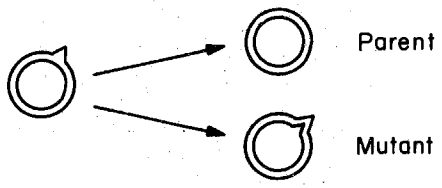


A synthetic oligonucleotide primes synthesis of a new strand of DNA on the template of the parental strand. In this case, the synthetic oligonucleotide contains a mismatched base pair, C where there should have been G. This will eventually create a mutation from serine to threonine in the protein encoded by this specifically mutated DNA.

which will accordingly be an exact complement of the parent everywhere except at those sites where the synthetic oligonucleotide had, by design, non-complementary bases. There results consequently a double-stranded DNA, one of whose strands, the parent template, contains the message for the parent protein; the other strand



After completion of the new strand (as above), a heteroduplex plasmid will have been formed, one strand of which contains the information for the parental protein; the other strand carries the information for the new, mutant protein. The site of the mutation is indicated as ⌒.

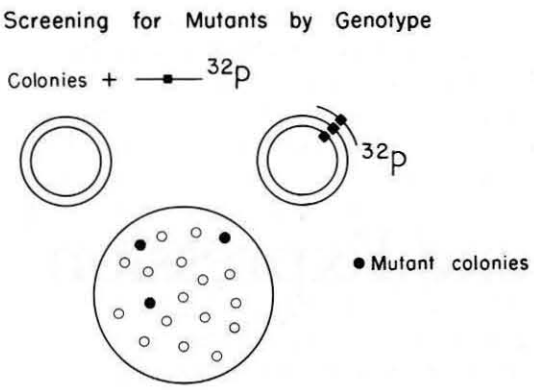


Normal semiconservative replication of the heteroduplex plasmid synthesized in vitro *as described leads to two types of descendants, parental and mutant.*

contains the message for the mutant gene whose DNA sequence reflects that of the synthetic oligonucleotide. These mutations can be conveniently created in structural genes that are constituents of circular, double-stranded, extrachromosomal plasmid DNA. Plasmids duplicate, as do chromosomes, before cell division; each daughter cell contains one copy of the chromosome of the parent and several copies of the plasmid. The information in the structural genes of the plasmid is translated into protein so that the cell synthesizes proteins encoded both by its chromosomal and plasmid DNA.

After this heteroduplex plasmid DNA is introduced into a suitable bacterial host, such as *E. coli*, the normal semiconservative duplication of double-stranded DNA will lead to two homoduplex descendants. One of these homoduplexes will be like that of the parent template; it will encode the original protein. The other homoduplex, however, will contain the base changes mandated by the mutagenizing synthetic oligonucleotide; it will encode a protein with altered amino acids at those sites specified by the base changes. Thus some clones of the bacterial hosts will contain the message for the parent protein, and some will contain the message for the mutant protein.

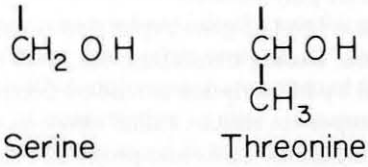
We now need to know which are which and could in principle decide by isolating proteins from many such clones and determining which produce mutant and which produce parent protein. But this would be an exceedingly inefficient approach. A much more elegant screening method is available that essentially allows us to "read" the DNA sequence of many clones very rapidly. Recall that the synthetic oligonucleotide will hybridize to the DNA of parental clones with one or more base mismatches (at the sites where the synthetic oligonucleotide purposely contained non-parental bases). In contrast the synthetic oligonucleotide will hybridize perfectly, with *no* base mismatches, to the DNA of the mutant clones. In hybridizing the mutagenizing oligonucleotide to the parental template to achieve mutagenesis, conditions of relatively low stringency were chosen such that extensive hybridiza-



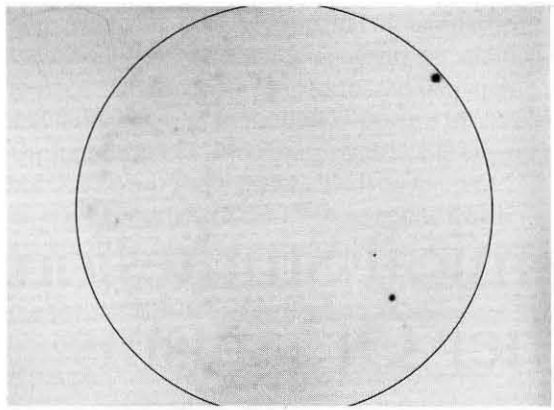
tion occurred even with 1 or 2 base mismatches out of a total of 15 base pairings (that is, 13-14 Watson-Crick base pairs and 2 or 1 mismatched pairs). For screening purposes, much more stringent conditions for hybridization are chosen that allow rapid and unambiguous identification of those clones that contain mutant DNA as compared to those clones that contain parental DNA, *even though the two differ in sequence by as little as a single base in many thousand*. Thus the mutant colonies can be identified, and from them the mutant protein can be isolated.

A recent spectacular application by Dr. Bruce Wallace and his group at the City of Hope of this ability to use the hybridization of a relatively short oligonucleotide to read the DNA sequence of genetic material has allowed the identification, by examination of their DNA, of those individuals who have either one or two genes that encode sickle cell, as contrasted to normal adult, hemoglobin. The genes differ from each other only in a single base. Such screening can be carried out on DNA obtained by amniocentesis and allows the determination early in pregnancy of a genetic defect as small as a single base alteration in the 6×10^9 base pairs that constitute the human genome.

The procedure to produce specifically mutated proteins has been developed at Caltech in collaboration with Dr. Arthur Riggs at the City of Hope and has been applied to the generation of mutants of β-lactamase, an enzyme that efficiently catalyzes the hydrolytic inactivation of penicillin and that is thereby responsible for the resistance to penicillin therapy of an ever increasing number of strains of infectious microorganisms.



The change of a serine to a threonine residue at the active site of β-lactamase leads to a complete loss of catalytic activity.



Under stringent conditions the synthetic oligonucleotide, labeled with radioactive ^{32}P, binds preferentially to those colonies containing mutant plasmid. When the plate is exposed to film, mutant colonies, having bound the radioactive oligonucleotide, appear as dark spots. The photograph represents a real experiment in which two mutant colonies were identified by this screening technique against a background of about 2000 colonies containing plasmid for parental β-lactamase.

In these mutants we altered serine residues to threonine residues in the active site of the enzyme. Serine and threonine differ from each other by only one carbon and two hydrogen atoms (as do methyl and ethyl alcohol). The normal enzyme has a ser-thr dyad at the active site. One mutant had thr-thr, that is, the serine was enlarged by a CH_2 group — a small increment of 14 daltons to a protein of 29,400 daltons. (A dalton is the unit used to express atomic mass.) This mutant is totally devoid of catalytic activity. Another mutant has ser-ser, that is, the threonine was reduced in size by a CH_2 group. This mutant has about 5-10 percent of the catalytic activity of the parent.

These are examples of the very few mutant proteins that have so far been produced by the technique outlined in this article, a technique that has been used to study the relation between protein structure and function for just about one year. So this approach is still in its very early days, and one can anticipate that the future will hold many fascinating surprises. Applications of this technique allow for the first time a truly systematic approach to the central question of the relation between structure and function in proteins and allow us to address such intriguing questions as: What are the essential structural features for a protein to function effectively as a catalyst, hormone, or receptor? How are the various structural aspects of an antibody related to its ability to distinguish self from non-self and thereby, for example, to initiate the destruction of infectious agents such as bacteria and viruses, or the altered cells that form tumors? What regions of a protein located on the surface of a cell carry the information essential to the interactions between cells that play a central role in the development from a single cell (the fertilized egg) of a complex organism such as man? From these studies will come striking new insights into the mechanisms by which proteins successfully carry out their myriad functions as well as the ability to design proteins with specific, novel, and useful properties. □

Biochemical Studies on Gene Expression in Higher Organisms

by Carl Parker

A chemical biologist is trying to gain an understanding at the molecular level of the mechanisms controlling gene expression

ONE OF THE MAJOR GOALS in modern biology and biochemistry is to understand exactly how gene expression is controlled in higher organisms. It is well known that genes are the basic unit of the material of inheritance. We know that each individual organism receives a set of hereditary information from its parent or parents, replicates it during growth or preparation for reproduction, and passes it on — usually largely unchanged — to its progeny. At present, there is very little understanding of how a single cell develops into a complex multicellular organism. It is very likely that the expression of genes at different times in different cells brings about, to a large extent, the development of a complex multicellular organism. Thus, the central question is, How is it that these genes are selected only at certain times in a select subset of cells?

My research interests are to gain an understanding at the molecular level of the mechanisms controlling gene expression. We know that there are several points at which gene expression can be controlled. The first level of gene expression, called transcription, involves the precise copying of the information contained in DNA (deoxyribonucleic acid) into another long polymer called RNA (ribonucleic acid). This process is mediated by a rather complex, multi-component enzyme called RNA polymerase.

The next level of gene expression is called translation. During translation, the RNA molecule produced by transcription is read by complex, multi-component entities called ribosomes to polymerize amino acids into proteins. Proteins in turn can be modified by other proteins in the cell, and thus a third level of the regulation of gene

expression is evident. In bacteria, where the molecular processes governing gene expression are becoming well defined, we know that the control of gene expression is effected at the level of transcription to a large extent. Thus, my research group is focusing its attention on transcriptional control mechanisms in higher organisms.

My research group currently consists of my research assistant, Joanne Topol; three graduate students, Warren Kibbe, Bette Korber, and Gil Scott; an undergraduate student, Doug Ruden; a postdoctoral fellow, David Price; and one technician, Terry Koch. We are taking a biochemical approach toward understanding transcriptional control mechanisms in eukaryotes. Our attention is focused on two organisms in particular: *Drosophila melanogaster* (a fruit fly) and *Saccharomyces cerevesiae* (bakers' yeast).

Drosophila is a particularly useful organism for biochemical studies of transcriptional control, since it possesses a group of eight genes that respond to the simple elevation of the temperature at which the fly is growing. These so-called heat-shock genes respond to elevated temperature by a dramatic increase in their levels of transcription and translation — to over a hundredfold more than they were at room temperature. The heat-shock genes, therefore, serve as an excellent model system for our studies aimed at discovering how genes turn on at the level of transcription.

Several other research groups have actually isolated all of the Drosophila heat-shock genes by using recombinant DNA technology. They have gone one step further to completely determine the DNA sequence (the sequence of nucleotides making up the DNA molecule and gene) of many of the heat-shock genes.

At Stanford, where I was a postdoctoral fellow with David Hogness, I developed a procedure for preparing RNA polymerase from Drosophila such that the enzyme would recognize the appropriate DNA sequence near a gene (a region called the promoter) and transcribe the gene correctly *in vitro*, that is, in the test tube. It was already known that a purified RNA polymerase from Drosophila was not capable of specifically starting transcription at the correct site *in vitro*. Thus, it was necessary to use a crude preparation of RNA polymerase in order for a biologically meaningful reaction to occur in the test tube.

My research group at Caltech is seeking to understand just what other proteins or "factors" other than RNA polymerase are required for initiation of transcription *in vitro*. It is our hope that by identifying these factors and learning how they function we will be better able to study the molecular processes that regulate gene transcription. We have, by a combination of approaches, identified three different components (in addition to RNA polymerase) required for the initiation and termination of transcription *in vitro* of several Drosophila genes. Increasing evidence is emerging that one of these factors binds to the promoter, a sequence of the DNA template that is known to be critical for transcription. The other two components are not as well characterized, but the preliminary evidence leads us to speculate that one of these two components interacts with the RNA polymerase. In fact, we have observed, by various biochemical procedures, that a complex of all three factors and RNA polymerase can be discerned. Should the transcription complex that we see in the test tube exist in the cell, one may conjecture that this complex is capable of initiating transcription on a wide variety of genes, provided the gene has either the appropriate structure or necessary accessory proteins to make that gene active.

In addition to the perhaps more general transcription factors I have just described, Joanne Topol and I have identified another factor that possesses a very interesting property. This protein(s) binds specifically to a DNA sequence that is known from experiments in other laboratories to be required for the transcriptional regulation of one of the heat-shock genes of Drosophila. This is a particularly exciting result because it allows us, for the first time, to begin experiments aimed at exploring the mechanism of transcriptional activation of a gene from higher organisms.

The next step beyond these experiments will be to actually isolate the genes for the more general transcription factors and the specialized heat-shock regulatory site binding protein. This can be achieved by several approaches including the advanced technology developed in Leroy Hood's laboratory involving the synthesis of a particular region of the gene based on its determined amino acid sequence. It is easy to imagine that we can at that point ask detailed questions about the regulation of gene expression of genes whose products control expression of other genes — thereby stepping up in the hierarchy of control of gene expression.

The facilities in the Braun Labs are not going to make our work any less complicated or demanding, but it should be physically easier to accomplish our research goals because of the excellent resources available there. We are fortunate also to be supported financially by the National Institutes of Health, the Camille and Henry Dreyfus Foundation, the Sloan Foundation, and the generosity of the Division of Chemistry and Chemical Engineering at Caltech. □

How to Find Needles in Haystacks: Fluorescence-Activated Cell Sorting

A new instrument in Braun Laboratories is a dual-laser fluorescence-activated cell sorter — an irreplaceable tool for isolating rare cells from mixed populations or for determining which properties are correlated in populations of cells that differ in many ways

by Ellen Rothenberg

THE TISSUES of higher organisms are composed of extremely heterogeneous populations of cells. Within one organ, the developmental origins of various cells can be quite distinct. A good example is skin, which is peppered throughout with surveillance cells of bone marrow origin, and which is colored by pigment cells that are related to nerve tissue. Even when the cells in an organ are closely related, they may carry out very different functions involving distinct biochemical pathways. Thymus-derived lymphocytes, for example, are strictly specialized, either to secrete growth-stimulating polypeptide hormones or to bind and kill target cells. Finally, the cells from a single clone, although developmentally equivalent, may show different properties in different phases of their growth. Actively dividing cells not only have more stringent nutritional requirements than resting cells but also become vulnerable to a variety of poisons and serve frequently as preferred targets for viral infections. Most dramatically, early cancer cells may differ from their normal homologues in very few properties other than growth rate.

To understand the mechanism by which an organ carries out a particular function, we must be able to dissect out the key subpopulation of cells and distinguish its responses from those of other cells. It is to make this possible that fluorescence-activated cell sorting has been developed.

The cell sorter carries out two tasks. First, it records differences among individual cells in a population on the basis of one or several correlated properties. Second, it physically isolates the cells with properties of interest from the cells in the rest of the population. While its ability to deliver purified cell preparations is its most dramatic tour de force, the cell sorter is also an analytical instrument of unparalleled power and flexibility.

The basis on which the sorter discriminates between cells is optical. Individual cells intercept a slit-focused laser beam and scatter its light. The intensity of light scattered "forward," in a halo around the laser target, is mainly dependent on the cross-sectional area of the cell. Light scattered at a 90° angle is more a function of the refractive index of a cell, of its granularity or quantity of intracellular organelles. Simply using the intensities of forward and 90° light scatter makes it possible to discriminate between lymphocytes, monocytes, and granulocytes in human peripheral blood samples.

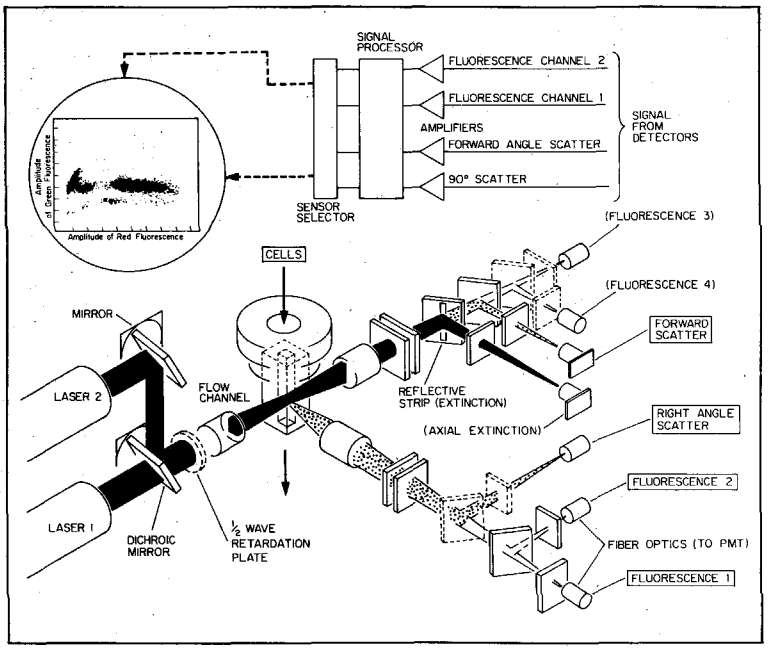
By using lasers tuned to appropriate wavelengths, it is possible to gather much more information. Cellular enzyme activities can be assayed with fluorogenic substrates, and cell surface molecules can be bound by fluorochrome-conjugated antibody molecules. The laser can emit at an excitation optimum for the fluorochrome (488 nanometers to excite green fluorescein, 568 nanometers for red tetramethyl rhodamine), so that the fluorescence intensity provides a fairly quantitative measure of enzyme activity or surface antigen expression in each cell. In the system installed in the Braun Labs, every cell is identified by up to four parameters, so that forward light scatter, 90° light scatter, and reaction with both green and red fluorochromes can all be considered simultaneously. In practice, this means that surface antigen A can be measured with a green-fluorescent antibody to A and surface antigen B with a red-fluorescent antibody to B. Then we can focus, for instance, on the largest, most granular cells in a sample and determine what fractions of that subpopulation are A+B+, A+B−, A−B+, or A−B−.

The sorter can analyze cells individually because of its fluidic system. Cells in dilute suspension are pumped from a reservoir into an optical flow cell, where they are funneled into a narrow stream and held centered by the flow of surrounding sheath fluid. This ensures that each cell will cross the path of the laser in the flow cell and makes it unlikely that two cells will coincide unless they are bound together. The light scatter and fluorescence signals from each cell are amplified, digitized, and fed immediately to the multichannel analyzer, so that within 40 to 120 microseconds a decision can be made on whether or not to collect that cell.

After traversing the optical flow chamber, the stream of cells and sheath fluid emerges through a vibrating nozzle that breaks it into droplets. Only one droplet in ten is likely to contain any cell at all. The droplets then pass between two deflection plates which are charged transiently if the command is given to sort. Two sorted populations can be collected by deflecting to the left or to the right, while unsorted droplets fall into a mass collection vessel in the center. The system can process up to 2000 cells per second without compromising the purity of the selection. Furthermore, living cells usually emerge from the instrument with undiminished viability and functional potential. Thus, in a typical preparative run of a little over an hour, we can collect a million cells of a type that make up one-tenth of the starting population.

The Cell Sorter In Genetics

One straightforward application of the sorting technology is to isolate extremely rare types of living cells. If the purified cells can maintain their properties and proliferate extensively in culture, then only relatively small numbers need to be collected initially. This application has been most useful in cases where the differences between the desired cells and the majority are genetic, and hence are differences that are maintained through many cell divisions. Classic somatic cell geneticists have used sorting to isolate rare mutant cells that express a new surface antigen. More recently, sorting has become a promising adjunct to recombinant DNA technology. If a cloned gene encodes a surface antigen or an enzyme that can use a fluorogenic substrate, then expression of the cloned gene can be measured by fluorescence. Thus, when the gene is introduced into cells under different conditions, the sorter can provide a quantitative comparison of the resulting levels of expression. Moreover, live cells expressing the gene can be isolated for further analysis and manipulation. This approach has been valuable both for studies of gene regulation and for identification of genes that encode particular cell-surface glycoproteins.



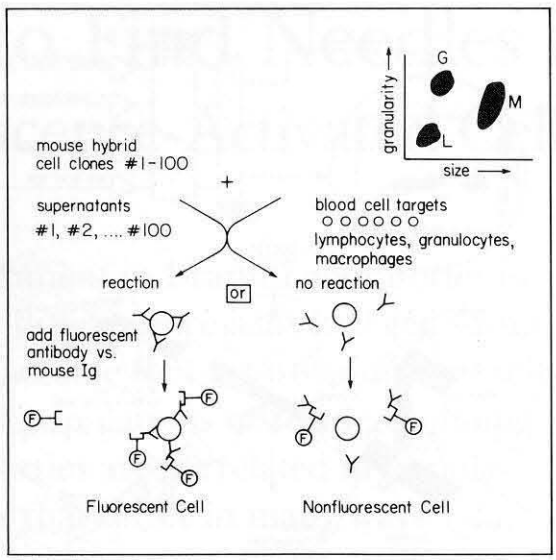
Optics of the cell sorter. Light scattered in a forward direction indicates the size of the cell, while the intensity of light scattered at a right angle from the incident laser beam depends on the cell's granularity. Green or red fluorescence detectors can measure the excitation of fluorescein and rhodamine, respectively, and can be used in either position.

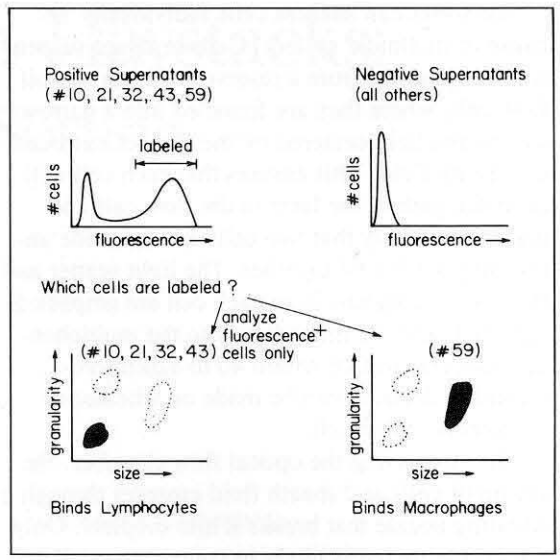
The Cell Sorter In Immunology

A different area where cell sorting has played a prominent role is the characterization of monoclonal antibodies. Monoclonal antibodies are homogeneous immunoglobulin molecules that bind specifically to one antigenic target. They are secreted by individual spleen lymphocytes that have been immortalized by fusion with lymphoid tumor cells. To make monoclonal antibodies against a desired cell type or a desired protein molecule, mice or rats must first be immunized with the cell or protein antigen. Spleen cells from the immunized animals are then fused en masse with the tumor cells, and hundreds of clones of fused hybrid cells are grown in culture. Each clone secretes some type of monoclonal antibody, but only a few will secrete antibody that binds to the target of interest. Finding those clones requires a massive and sophisticated screening program.

Cell sorting has provided two kinds of useful screens. First, a protein antigen can be used to coat fluorescent microspheres, which then bind only to those hybrid cells that make antibody to the protein. The hybrids with bound fluorescent microspheres can be isolated directly by the sorter. Second, when the antigen of interest is a particular cell type, the sorter can be used analytically to measure binding of each of a panel of monoclonal antibodies to that kind of cell. The antibodies secreted by different hybrid clones are separately reacted with the target cells, and

Identifying antibodies reactive with a particular target cell type. The monoclonal antibodies secreted by 100 different hybrid mouse cells are each allowed to react with blood cell targets. Fluorescein-conjugated antibody against mouse antibody makes a fluorescent "sandwich" wherever a mouse antibody succeeded in binding. The cells bearing the fluorescent complexes are analyzed in the sorter. Antibodies 10, 21, 32, and 43 all bind lymphocytes. Antibody 59 binds to macrophages. If anti-lymphocyte antibodies are desired, antibody 59 is discarded.



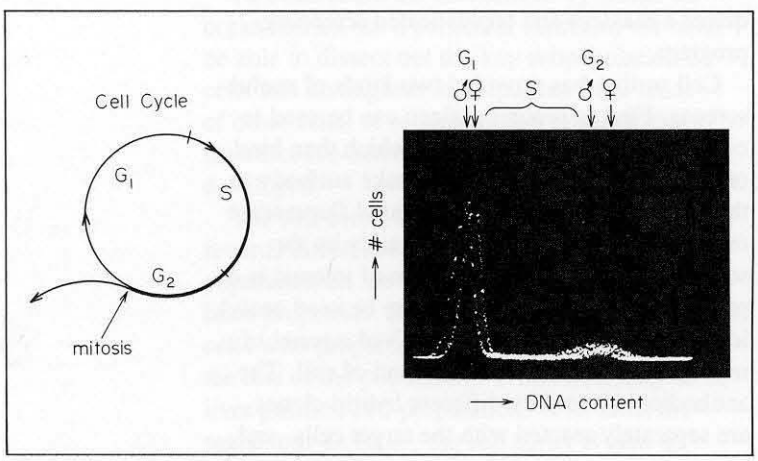


binding is detected by adding a fluorochrome-conjugated rabbit antibody against mouse or rat immunoglobulin (illustrated above). Because the sorter can measure additional properties of the target cells, it can discriminate between binding to the targets of interest and binding to some other cell type or to debris that may contaminate the target sample.

The Sorter in Cell Biology and Physiology

In addition to these applications, the cell sorter provides a unique approach to studies of growth control. This stems from the availability of fluorescent dyes that bind stoichiometrically to DNA in chromatin. The fluorescence recorded from stained cells is directly proportional to the DNA content of the cells. Thus, in histograms of cell number vs fluorescence intensity, one can readily distinguish the peak of cells that have replicated their DNA prior to mitosis (G_2) from the cells that have not yet begun DNA synthesis (G_1) since their last division. As shown in the figure below, even subtler differences are discernible, including the difference between male and female mammalian cells. The Y chromosome of the male is much smaller than the X chromosome, so that each male (XY) cell nucleus contains slightly less DNA than the female (XX) nucleus at a corresponding stage of the cell cycle.

Distinguishing male and female cells by DNA content. The ordinate shows the number of cells with a given intensity of fluorescence. The abcissa shows increasing fluorescence intensity, a direct measure of DNA content. G_1 cells have recently divided and have not begun to replicate their DNA for the next division; G_2 cells have just finished copying their DNA prior to division. Male cells at each stage have less DNA than female cells because the Y chromosome is smaller than the X chromosome.



Most determinations of cellular DNA content are carried out on fixed, permeabilized cells, because most of the DNA-binding dyes do not penetrate viable cells. One of the most interesting recent developments, however, is that new DNA-binding dyes have been found that enter live cells without serious toxic effects. This has opened up the possibility of sorting cells on the basis of their behavior.

Most cells in mammalian organs are in a resting state, and a crucial determinant of their function is often the type of stimulus they require to initiate DNA synthesis. The cells that respond to a particular signal in this way can be isolated by sorting on the basis of their increased DNA content. Even if the cells are fixed, the sorter can provide correlated information on their surface antigens and size. Since responsiveness is essentially a behavioral characteristic, though, it is most interesting to preserve these cells' viability, to be able to establish them in culture where their responses can be analyzed in greater detail.

These examples illustrate the cell sorter's broad range of analytical and preparative applications. In addition to the statistics it provides on the characteristics of cell populations, it can physically isolate rare cells defined by unusual combinations of those characteristics. Almost any property that can be measured optically may be exploited to distinguish between subclasses of cells. The limits of the sorter's versatility are mainly determined by our ingenuity in devising fluorescence and colorimetric assays and by our imagination in using them. □

Research in Progress

Good Vibes

LIKE A BELL or a violin string, the earth vibrates in a discrete number of frequencies when it is "rung" or jarred by an earthquake. Theorized in the 19th century, the existence of these long-period free oscillations was not proven until the 1950s, when Caltech's Hugo Benioff developed a seismograph capable of measuring such long-period motions and, with some surprise, observed long-period vibrations that he thought were excited by the 1952 Kamchatka earthquake (magnitude Mw = 9.0).

He found on his record oscillations having a period of 58 minutes, which had been predicted by theoretical calculations as the period of the gravest (lowest in "pitch") of these oscillations. The earth's free oscillations were observed beyond any doubt by Benioff, Frank Press, and Stewart Smith after the 1960 Chilean quake (Mw = 9.5), and for this event they also first used the amplitude and phases of the oscillations to determine the properties of the earthquake source — the fault length and rupture velocity.

In the 20 years that followed, analysis of the earth's free oscillations with increasingly refined techniques has provided much information on the source mechanisms of earthquakes and on the earth's interior structure. During the last decade worldwide seismic networks (IDA — International Deployment of Accelerometers, and GDSN — Global Digital Seismographic Network) have furnished high-quality, digital data on the earth's "music" that is scanned, analyzed, and stored by computer, and made available to scientists studying the phenomenon.

One of those scientists is Hiroo Kanamori, professor of geophysics, who has made a number of contributions to the field, both in the development of new techniques and their application to the characterization of earthquake sources. His recent work on long-period surface waves (those oscillations of periods shorter than 300 seconds and wavelengths less than 1500 km) took an unexpected turn when he observed, again with some surprise, that the volcanic eruption of Mount St. Helens in May 1980 excited these waves, which were recorded by IDA and other networks. Since a volcanic eruption had never been observed by global seismological networks before, the event provided some unique data and an interesting picture of a volcanic source mechanism.

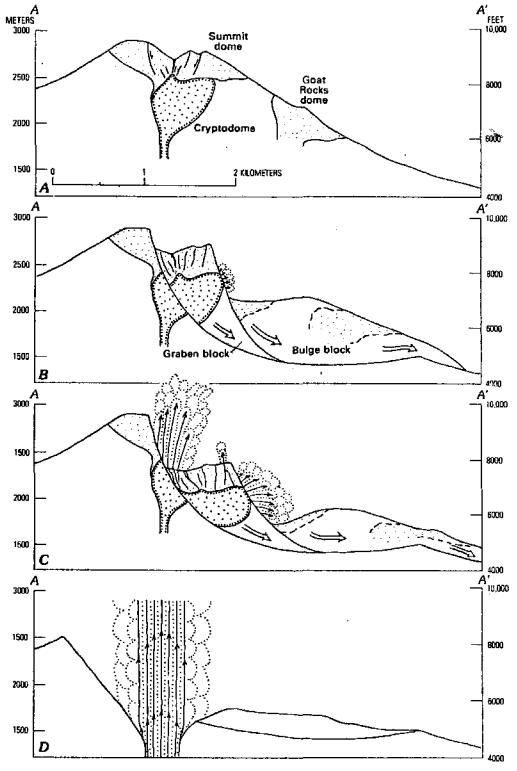
Kanamori and graduate student Jeffrey Given found that the source could be represented by an almost horizontal single force pointed in a S5°W direction and that the peak value of the force was about 10^{18} dynes. This is approximately equal to gravitational force acting on a conical mountain with a base diameter and height of about 1 kilometer. They also found that it was a relatively slow source process, much slower than ordinary earthquakes. At first they were not sure exactly what caused the seismic signal but concluded from the magnitude, the geometry (or direction), and time history of the force that the source was the massive landslide that touched off the eruption. The initial lateral blast (different from the vertical blast seconds later) may also have contributed to the horizontal force.

As magmatic activity increased the pressure inside Mount St. Helens, a bulge began to raise the incline of the north face of the mountain. The increase in slope led to the massive landslide down the north face (producing the southward force on the ground as it accelerated), removing the pressure near the vent and releasing the blast.

It has been generally thought that a magnitude 5.2 earthquake triggered the landslide, that the north slope was not sufficiently destabilized by the bulge to fail, but Kanamori now theorizes that the giant slide was caused by the instability of the slope and was responsible for the seismic signal. He suggests that there may not have been an ordinary earthquake at all, but only the force created by the removal of such a large mass from the mountain (a total of 5×10^{15} grams of material from the slide and the blast). Although the mechanism of the Mount St. Helens eruption is still not known precisely, interpretation of the earth's vibrations has added a new dimension to understanding the basic physics of the process.

Kanamori and Given have also developed methods of using long-period surface waves for rapid evaluation of an earthquake's tsunami potential. Tsunamis (tidal waves) are caused by deformations of the sea floor, primarily by earthquakes with a dip-slip rather than a strike-slip motion. Since surface waves travel faster than tsunamis, the Caltech scientists' method for retrieving a quake's fault geometry from global network data within minutes would make predictions and warnings of tsunamis possible. The system, according to Kanamori, would be relatively easy to implement. □ – *JD*

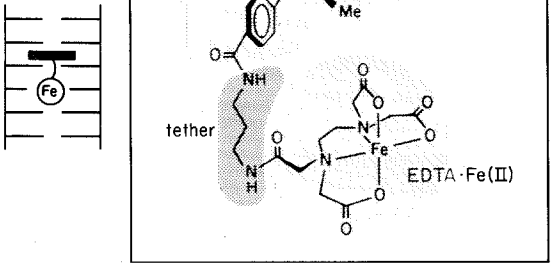
From *The 1980 Eruption of Mount St. Helens, Washington*, Professional Paper 1250, courtesy of the United States Geological Survey.



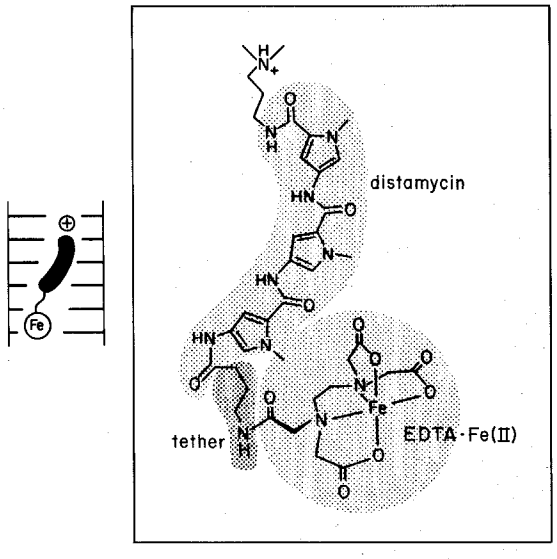
In the weeks preceding the eruption of Mount St. Helens, the increasing magmatic pressure (cryptodome) had caused the summit to split and a bulge to expand northward, lifting up the mountain's north side (A). The landslide that initiated the eruption removed the north face in two sections (B), exposing one side of the cryptodome and causing the lateral blast. As it slid farther (C), another explosion was triggered from the now exposed top of the cryptodome, and the vertical blast 20 km into the air followed (D) as the main volcanic channel was uncovered. Hiroo Kanamori's analysis of long-period surface waves suggests a new interpretation of the seismic signal excited by these events.

Drug Footprints

THE BINDING of small molecules and proteins on specific sites along double helical DNA is important in the regulation of many biological processes. For instance, many antibiotic, antiviral, and antitumor drugs useful in chemotherapy are small molecules that bind to DNA. To solve the problem *where* such drugs bind on the DNA template, Professor of Chemistry Peter B. Dervan and his students set out to design a new DNA-binding and DNA-cleaving molecule, whose function is that of a sequence-neutral DNA scissor. Their success in this effort has led to a rapid, direct technique for determining the locations and binding site sizes of small molecules bound on heterogeneous double helical DNA. Moreover, their design strategy has allowed them to create sequence-specific DNA-cleaving molecules that might be useful as new antiviral and antitumor drugs, as well as new powerful tools for the manipulation of DNA.



***MPE**•Fe (II) consists of the flat methidium molecule joined by a tether to the iron chelator EDTA. Methidium binds to the DNA ladder (left) by sandwiching itself between the base pairs. The attached EDTA with iron in its center acts like a wrecking ball to break the DNA.*



The banana-shaped antibiotic distamycin binds to DNA at a four-base-pair site by fitting into a groove of the double helix. By tethering the EDTA wrecking ball to the distamycin, the DNA can be cut at the specific site.

Their source of inspiration was bleomycin, a glycopeptide natural product used as an antitumor drug in man. Bleomycin binds a two-base-pair site on the DNA chain, and in the presence of iron and oxygen cleaves at that site. Because bleomycin is a complex structure, the details of the binding and DNA cleaving are still poorly understood by researchers. Bleomycin's biological activity is presumed to be related to the DNA cleaving event.

Dervan and graduate student Robert Hertzberg mimicked bleomycin's efficient DNA-cleaving activity by connecting two molecules that each performed one of bleomycin's functions. Methidium, a flat, aromatic molecule that binds to DNA by sandwiching itself between the base pairs was linked by a tether to ethylenediaminetetraacetic acid (EDTA), a simple and well-known iron chelator. EDTA • iron acts like a "wrecking ball" and cleaves the DNA helix where the methidium is attached.

Although Dervan claims that the resulting molecule, (methidiumpropyl-EDTA) iron(II), or MPE • Fe(II), is only a primitive model of the much more "exquisite" bleomycin, MPE is quite efficient at what it was designed to do. And it goes bleomycin one better for Dervan's purposes in that it is *not* sequence specific. It will cut DNA at any set of bases, thereby mimicking in function a DNA-cleaving enzyme called DNase I. But DNase I can be very sensitive to DNA structure (conferred by different base sequences) so its use as sequence-neutral DNA scissors sometimes suffers from the lack of complete non-specific DNA cleaving.

Non-specific DNA cleavage makes MPE a new useful tool to determine drug-binding sites on the DNA template. The rapid technique developed by graduate student Michael Van Dyke is called "footprinting with MPE•Fe(II)." When small molecules bind DNA, they protect that site, so that when the MPE•Fe(II) scissor happens along it can't cleave it where that drug is sitting. When DNA strands of known sequence are radioactively labeled on one end and a very small amount of MPE•Fe(II) is added, so that it cuts each strand just once, the resulting segments can be analyzed on a Maxam-Gilbert sequencing gel. Since MPE•Fe(II) will cut anywhere that the drug has not bound, when the ladder of base pairs is observed on the sequencing gel, there will be gaps where the drug has bound. This DNA-cleavage inhibition pattern is called the drug's "footprint," and while footprinting had been done previously with protein-DNA-binding sites, Dervan and Van Dyke are the first to adapt it for small molecules. Thus far they have used this rapid and direct method for identifying the DNA binding sites of seven drugs — actinomycin D, distamycin A, netropsin, chromomycin, mithramycin, olivomycin, and echinomycin. Many of these molecules are important in antibiotic, antiviral, and anticancer chemotherapy. A knowledge of where drugs bind on DNA is one step forward in understanding the molecular basis of antitumor, antibiotic action.

With the knowledge of these binding sites on DNA and the proven efficiency of his attachable "wrecking ball," the Dervan group was then able to construct molecules that would cleave DNA sequence specifically. The goal is to design and construct DNA-cleaving small molecules that would act like restriction enzymes, proteins that recognize and cleave DNA at sequences four to six base pairs in size. The ability to cut and paste DNA at a particular site in DNA is the basis for much of genetic engineering. The longer the recognition sequence, the less often it occurs and the more precisely the target can be defined in the DNA polymer, which is thousands to hundreds of thousands of base pairs long. By equipping the antibiotic distamycin with EDTA•Fe(II), graduate student Peter Schultz and postdoc John Taylor successfully constructed a molecule they called distamycin-EDTA that binds and cleaves at a specific four-base-pair site, rich in adenine and thymine. This same strategy can be used to construct other molecules that can recognize DNA sequences four to six base pairs in size, creating in effect a new set of "artificial restriction enzymes." Dervan envisions the construction of opposite strand DNA-cleaving agents at defined sequences as large as 8 to 16 base pairs. Once that happens, chemists will have gone beyond the sequence specificity nature has provided, affording a new class of useful DNA-cleaving machines for site-specific cleavage of viruses and chromosomes. □ – *JD*

Oral History

Pol Duwez — How It Was

Pol Duwez, professor of applied physics and materials science, emeritus, is one of the world's leading scientists in the field of metals and materials. He has conducted research leading to the development of a series of new alloys that are widely used in industry, and he pioneered in work that led to the discovery of metallic glasses. Most recently he has been involved in research on metals known for properties of superconductivity.

All this work has been recognized by a number of awards, including in 1981 the William Hume-Rothery Award from the Metallurgical Society of the American Institute of Mining, Metallurgical, and Petroleum Engineers, Inc.; and the Heyn Medal of the German Society for Metals Science. He is a member of numerous professional societies and was elected to the National Academy of Sciences in 1972. For many years he was a member of several boards and committees of government agencies concerned with national defense.

Duwez held an appointment at the Jet Propulsion Laboratory in the late 1940s and early 1950s, and he has been a full professor at the Institute since 1952. In 1979, Harriett Lyle interviewed him for the oral history project of the Caltech Archives. E&S presents here an excerpt from the transcript of those interviews.

Harriett Lyle: What are some of the first memories you have of Belgium and what it was like to be a child there?

Pol Duwez: The first thing that I cannot forget is when Belgium was invaded by the Germans in 1914; I was seven years old, and I remember the mounted German soldiers, called Uhlans de la Mort, with a skull as an insignia on their shakos. Before that, I remember that I went to the opera to see *Faust* in Brussels.

HL: Was your family interested in music?

PD: My father and mother appreciated music, but they never played any instrument. But my older brother became a professional cellist in an orchestra. He taught me when I was six, and at seven I had a quarter-size cello. I wish I could have kept it, but we lost everything when we left Belgium at the beginning of the Second World War. When I was twelve, I got my first prize in cello at the Mons Conservatory in my home city, playing the "Davidow Concerto."

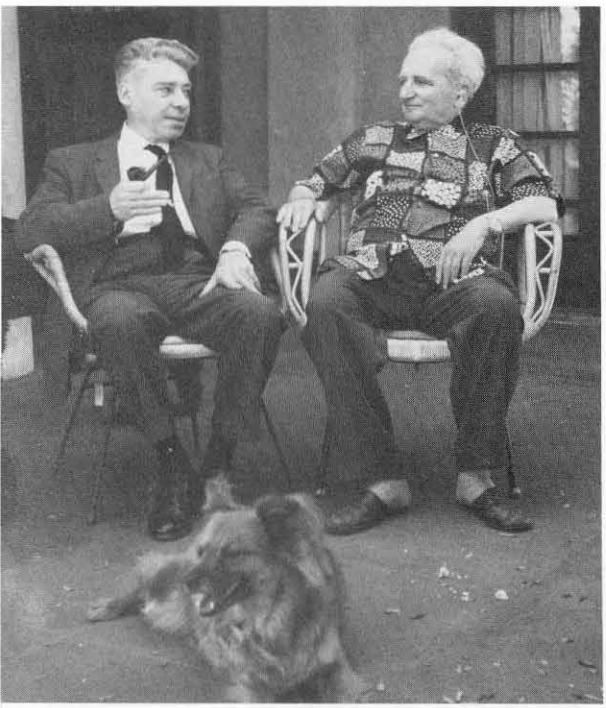
HL: Do you remember any interest in science or exposure to it?

PD: When I was in high school, I was mostly interested in physics and mathematics. I don't know if I could have made up my mind between the two. Then came the question of continuing to university. At that time in Belgium we had four universities — Ghent, Liège, Brussels, and Louvain. But there was also a School of Mines and Metallurgy Engineering that was famous in Mons. Now the question of going to the University of Brussels for science was a question of cost and distance. It was only 60 kilometers, but that meant time and money to go to Brussels and back home. So I decided I would stay in Mons and go to the School of Mines. Fortunately, the first two years there were just comparable to what you would have in science at the university. In the third year I started to study, in addition to the courses in Mons, what would be required for the science curriculum in physics and mathematics. It was possible to do this at that time. You could go to a central examining committee to be examined on what would be required to have a degree in physics in any university.

HL: I'd like to talk a little about how you as a child experienced the occupation of Belgium by the Germans.

PD: The main point, as far as I remember, was that we were lacking in food, but fortunately Herbert Hoover, who became president later, created the Commission for Relief in Belgium, and he got plenty of money from American charity. It was very well organized, and we started receiving food regularly from this country. In school, for example, we got a small piece of bread every morning to supplement what we did not have at home before leaving for school.

That particular commission had surplus money when the war ended, and that surplus became a foundation, the Commission for Relief in Belgium Educational Foundation, which later became the Belgian-American Educational Foundation



Pol Duwez with Theodore von Kármán in 1961. Chien, the dog, belonged to Duwez.

and which awarded the fellowship that I received in 1933 to come to Caltech.

HL: When you went to the School of Mines, were you interested in the mining profession?

PD: No. The school was both mining and metallurgy, and I was interested in metallurgy.

I graduated in 1932 as an engineer, and I left for the United States with a fellowship. I went to the University of Michigan as a graduate student because I wanted to work with Professor Timoshenko, whose field of interest was mechanical engineering, the strength of materials, and so on.

As in any American university, you had to pass a physical examination, which was much more detailed than it was in Belgium, where I had passed the physical examination with the American foundation which was sending me over here. In Michigan it was a mass production, thousands of students going through the line for X rays. And the results of my chest X rays came out badly, and they claimed I had tuberculosis. That created a big stir in Belgium, because the foundation had sent me after a thorough physical examination. The foundation sent me back to Belgium because I could not continue at Michigan. After several months, they decided it was a mistake. They had assumed that the spots on my lung, which were scars from pleurisy that I had had earlier, were active tuberculosis.

In retrospect, it was a very happy circumstance that they sent me back to Belgium, because in one year I got my doctor's degree in physics at Brussels. When I came back in 1933, I came to Caltech because of Professor von Kármán, who was in aeronautics and very much interested in solid mechanics.

Since I had enough financial support, I had no problem being accepted at Caltech as a research fellow, because they didn't have to pay me. They opened the door, and when I went to see von Kármán, we discussed problems and I started to work immediately. Well, it was a combination of von Kármán and Fritz Zwicky, who was at that time interested in solid state. Later on he became an astrophysicist. But he was interested in the plasticity of metallic crystals, which was a big subject at that time. The field was in a transition between empiricism and real understanding of what was happening. Zwicky and von Kármán were a very good combination for me. I learned quite a bit from my collaboration working under these two excellent men.

HL: When you came here, it was in the middle of the depression in this country.

PD: I did not feel the depression, the reason being that my Belgium fellowship was very generous in giving me $130 per month. With a roommate, I had an apartment on South Hudson Avenue with a living room, kitchen, and two bedrooms for $15 each. Eating out twice a day, including a steak dinner, amounted to about $1.60 a day. The rest — laundry and so on — was minimum. At the end of that first year at Caltech, I managed to save enough money to take a trip around the world. Transportation, by ship obviously, was arranged by the Japanese N.Y.K. Line for a total of $550. I spent ten days in Japan — then went to Shanghai, Hong Kong, Singapore, Penang, Ceylon, and back to Belgium to see my family. Then I came back to the United States. For all that I spent less than $650.

So I did not feel the depression, but some people were really in bad shape. I remember on the corner of California Boulevard and Lake Avenue there was a filling station, and the man who used to serve gas on my old Model-T that I bought for $10, by the way, had a master's degree in geology. He could not find a job in any field of geology.

HL: When you got back to Belgium in 1933, did you know then that the Germans were preparing again for another war?

PD: In 1933 — yes. Between 1933 and when my wife and I finally left in 1940, it was obvious that something was going on, that sooner or later we would be involved, in spite of the fact that people just could not believe that there would be another German occupation. Well, it did happen. But it was really obvious only a few months before the invasion started. In fact, we were caught there after the Germans were already halfway through Belgium. My daughter, Nadine, was about three years old. We put everything in the Ford we had and took the highway to southern France where my wife had family. We were trying to get as far away as possible from the invading German army, but already they were bombing everything and machine-gunning people on the road.

My wife was an American citizen, although born in France, but naturalized. My little daughter was considered an American citizen because she was registered at the American embassy in Brussels, where she was born. So they could immediately get their sailing pass and be admitted to the United States. But I was not an American citizen, so I had to wait while my family came here to Pasadena.

HL: Had you met your wife in Pasadena?

PD: Yes. Through the Alliance Française. We were invited to see a French movie in a small theater here in Pasadena by a friend who was a professor of geology here at Caltech. My wife had taught in the Los Angeles high school system before, so some of her friends helped her and she got a job with that system in 1940.

I had some difficult times in southern France. I was not supposed to stay there because the Germans had an agreement with the Petain government that all the Belgian people, British, and so on would go back to their own countries. I was with my wife's parents in a small village in southern France, and I knew the gendarmes so I could stay there and nobody said anything. The only trouble came when I was taking a train to go somewhere — like to Marseilles to the American consulate — coming out of the station there was a control established by the French, but under German pressure, to find out if some of the people like me didn't have the right papers. I always managed, but I had to wait six months for an American visa, which my wife

arranged in Washington with the help of the Hoover Foundation. By the time the visa came, I had no money left, but I received a mysterious check from Lisbon from a man who had been the prime minister of Belgium. I understood later that he got a request from the American foundation to send me money to go by train from Nîmes to Lisbon. The money enabled me to buy a ticket as far as Madrid, but by then I was short of money again.

You know, the best way to find out about something that is underground is to go to a cafe and ask the waiter if he can help. So I asked a waiter where I could sell gold. He told me, "Go to a building on such and such a street; go to the third floor; knock on the first door to the left, and if somebody answers, tell them what you want to do." So I went there, and there was a very mysterious-looking man with a balance scale. I had a few small things in gold — a watch, a chain, the souvenirs I received for my first prize in cello, for example. He put all of them on the balance and was telling me they were worth so much in Portuguese money. When he reached the number that I had to pay for the ticket, I said, "That's enough." But he had taken practically everything I had. It was nine o'clock in the morning, and the train was leaving at seven or eight at night. I didn't have any money to buy food, so I waited in a public park with my cello and two suitcases. At Lisbon, nobody was waiting for me, but I had word that I could pick up money at such an address. The Foundation, again, had arranged to give me money. So I got a big meal in Lisbon.

HL: I would like to discuss the direction you were going in science — at Caltech and back in Belgium in the period just before the war.

PD: The first year working with von Kármán and Zwicky, I was more or less continuing what I had done in my thesis in Belgium on the plasticity of metallic crystals. It was just the beginning of studying the deformation in metals based on single crystals instead of polycrystalline materials like all metals are. So the information was getting more fundamental. The incentive behind it at that time was that the theoretical physicist can compute the strength of a pure metal based on the atomic bond and some approximations. But when you measure the strength of the pure metal, it is less than one-thousandth of what it should be. So there is a large discrepancy between what is calculated on the theoretical basis and the actual observation that the material starts deforming much sooner at much lower stress. The approach was that we should check that situation on a single crystal, not a polycrystalline one, because that makes the problem closer to the theoretical value. Well, it was even worse. What was the reason?

The first papers that gave an explanation were published when I was here by Taylor in England. It was shown that the theory assumes a perfect crystal, a perfect arrangement of the atoms into a periodic field. And that is not the case. The real crystal is far from perfect, and — due to the theory of Taylor — it will be very much weaker. But Zwicky was fighting for his idea that the most stable form of the crystals is the imperfect one. It was found out later on that he was not right, but he defended it to the end.

I went back to Belgium in 1935 and was with the Center for Scientific Research there, which is equivalent to the National Science Foundation. Then in 1937 I became director of the National Laboratory for Silicates in the School of Mines in Mons and stayed until 1940. I was put in charge of a new lab working in the field of ceramics and had a chance to start from zero, buy all the equipment. One of the first small pieces of equipment I bought was a Beckman pH meter.

HL: In 1940 did you come directly back to Caltech from Lisbon?

PD: I came back to Caltech and, of course, the first thing I had to do was to go and see Dr. Millikan. He was a remarkable man, familiar with everybody on the staff at Caltech. So he recognized me immediately. He said, "You are very welcome back to Caltech, but I'm sorry we don't have any money to pay you." I said I was not asking for money, and all I wanted was a place to work. He said, "We'll find you a desk somewhere and a room." So I went to see Professor von Kármán and Professor Donald Clark. Von Kármán gave me a problem to work on, and I could use the equipment that Clark had in the basement of Throop Hall. The problem had something to do directly with defense. It was obvious that the United States would be involved in the war sooner or later.

The problem had to do with the resistance of a building or a ship to an exploding charge. So it comes back to the resistance of solids to a rapidly applied load in the milliseconds range. It was known that under these conditions an elastic wave propagates through the metal, but beyond a certain load the material must deform plastically. I suggested I would perform some experiments using Dr. Clark's equipment to find out what happens exactly.

That was in June. By October or November, I got my preliminary results, and they were already interesting. I wanted to communicate that to von Kármán as soon as I could. I think it was a Friday when I went to the Guggenheim building to show him the results, and I told him what I thought it could mean. He looked at it very hurriedly because he had to leave immediately for New York. I gave him all my rough notes, just a few graphs, and some scribbling of calculations. He took everything. Exactly three days later, he sent me a short letter, pencil-written on New York Central Railway stationery. This letter contained the entire theory of plastic wave propagation. At the end, he says, "I hope this will fit exactly with your experimental finding." In fact, it did check very, very closely. He had the theory written in no time, in very simple mathematics, as usual for von Kármán.

HL: So you did an experiment, and then on the basis of that experiment he pulled the theory together?

PD: I think he had the idea of waves — or propagation of the deformation. But when he saw the shape of the curves, of the results, he immediately saw where they came from, theoretically. That is how his mind worked.

I made more experiments, and the paper was written, the theory by von Kármán and the experiments by myself. A paper was sent for publication to the *Proceedings of the National Academy of Sciences*. Not more than a few days later, he received a letter from Vannevar Bush, director of the NDRC [National Defense Research Council] saying he would request that the paper should be withheld because of its importance to defense. Well, von Kármán told Bush, "That's all right, but I have here a man who has worked six months on this program, did all the research, and has not received any salary. NDRC should support this research with a contract." Bush agreed, but I was not a citizen, so my report was stamped confidential, and I could not receive a copy because I was not cleared. But from there on, I received a salary, and Caltech received a contract. I think the

salary was $120 a month, which was all right at that time.

HL: Did you continue this kind of work for the rest of the war?

PD: No. I would not say the whole problem was solved, but it reached a point where we could not do much more. What made me change my field again was the information received by the Guggenheim Laboratory people about the German missile — the V-2. That information came here to von Kármán, and the Guggenheim Aeronautical Laboratory (known as GALCIT) started an important project under the sponsorship of the Army Ordnance Department. That was the beginning of the GALCIT rocket research project which later became JPL.

Anyway, von Kármán called me at home one night, and he said, "Would you like to be in charge of a project concerning materials for rockets? We have a big problem, and we have to do something fast. We will probably build a lab to help you work on materials for jet-propelled rockets. We have a meeting tomorrow." I said, "Yes, I will do what I can." So that was the start of my involvement with JPL.

HL: You were going to work on the materials for the rocket itself, not the propellant?

PD: No, not the propellant. That was a chemical problem. He had people for that. But the materials for the combustion chamber must stand very high temperature. You cannot find any materials to stand it, but you can cool the chamber, and that's how it's done.

HL: Did you know how they did it with the V-2s?

PD: We knew they were cooling it. The combustion chamber was essentially ordinary steel, but it was very cleverly cooled. They were injecting some of the propellant to build up a liquid layer between the hot gas and the steel.

Eventually, we did some work at JPL developing alloys for high-temperature, long-time gas turbine blades. Also I originated research on cooling of gas turbine blades, because I was rather pessimistic about increasing the temperature above a certain level. After all, all metals have a melting point. So above a certain temperature, you cannot use a metal, and we worked on ceramics for the blades.

HL: Let's focus a little more on what you did as you began doing most of your research on the campus in the 1950s.

PD: The number of my students increased — slowly, but I got a chance to do some research leading to PhDs for some of them. The subjects became mostly concerned with the structure of new alloys, new metals. Titanium became a research subject, and we worked on the structure of alloys of titanium with other metals. Titanium and aluminum and titanium and vanadium became commercial alloys later on.

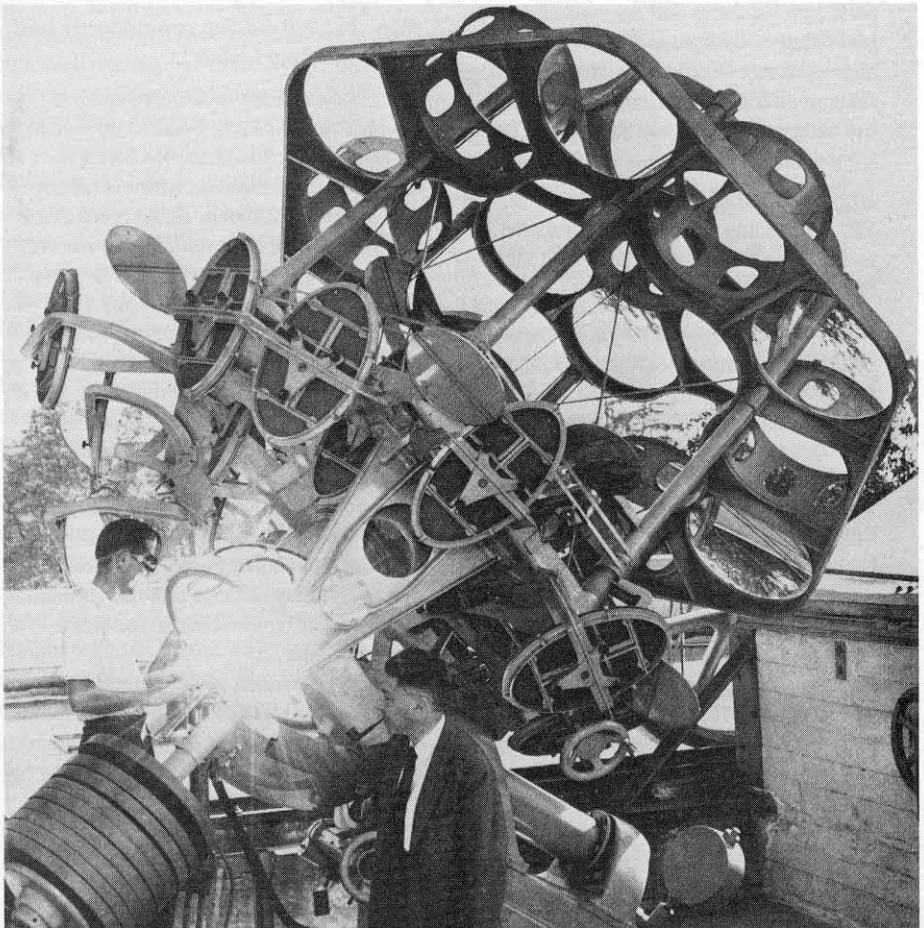
Molybdenum came along for use at high temperature. We worked on the so-called sigma phase, which was a very troublesome phase that causes brittleness of some high-temperature turbine alloys. We determined the structure, which turned out to be wrong, but we had prepared very good specimens. Gunnar Bergman [PhD '51], who was a student of Linus Pauling's in chemistry, got our specimens and with enough patience and hard work determined the right structure; and that was his thesis in chemistry.

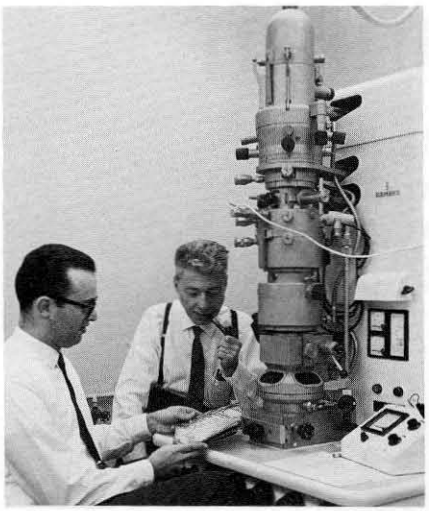
In the meantime, I went back to ceramics, which was my old subject in Belgium. We looked at ceramics made of the oxides of the so-called rare earths in the periodic table — gadolinium, neodymium, lanthanum, and so on. I always thought that these oxides with high-temperature melting points would eventually be useful. We built special furnaces going to 2200° centigrade and higher. The graphite furnace could go to 2500° C.

Now I don't know how many years later the question came back of studying these same materials that we made at that time. Why? Because they have a high electrical conductivity at high temperatures. If magnetohydrodynamics is developed practically, they will be used as electrodes. This is a typical case of pure research leading to something without knowing it — and also waiting a number of years before it becomes a practical material.

My studies of high-temperature oxides continued for several years after I left JPL, because I had access to the solar furnace located on top of Robinson Astrophysics Laboratory. The solar furnace was built in 1930 by George Ellery Hale, the astronomer, and designed by Russell Porter, who designed most of the Caltech telescopes. In 1932 that solar furnace was in operation. It consisted of 32 lenses, two

The brilliant flash in this 1956 photo of the solar furnace once located on the roof of Robinson Laboratory is the concentration of sun rays on a piece of refractory oxide in the focal area. With Duwez is Eugene Loh (MS '53, PhD '54), at that time a metallurgist with the Stanford Research Institute and now with Hughes Aircraft.





In 1965 Duwez and Ronald Willens (BS '53, MS '54, PhD '61), assistant professor of materials science, were using an electron microscope to check the structure of alloys made with the rapid cooling technique.

feet in diameter, focusing all the sun's rays in a single circular area about one centimeter in diameter. The total power concentrated in that circle was three and a half kilowatts of radiant energy. We could actually melt thorium oxide, so the temperature was about 3500° C. That was not every day, because this required a very clear sky.

HL: I'd like to discuss the evolution of your work — your ideas about science, how your work evolved and what caused those changes.

PD: The main reorientation of my research program came in 1959, when I started a new technique of quenching from the liquid state. By quenching, I mean cooling at extreme rates from the liquid state to bypass what happens during solidification or at least to modify the solidification mechanism. I had that idea as far back as 1956, trying to obtain new structures by passing rapidly through the liquid-solid transition. It took me several years to have a good idea on how to do it, because the classical methods would not apply. This idea became a very simple one. It was to spread the liquid metal as fast as possible — into a layer as thin as possible — on a very good heat-conducting surface, which of course was copper. I never thought it would be possible to completely suppress the crystallization at that time. I just wanted to modify the system, which was copper-silver, and the results were positive. We got a solid solution in the copper-silver system.

We continued the program with other systems analogous to copper-silver, but in which it was very improbable to find a solid solution. Then something else happened — a new crystalline phase that was not in equilibrium. Then, by analogy again, the next system we took was a gold-silicon alloy, very similar to the previous one. But instead of a crystalline phase came an amorphous phase. That was the first liquid-quench amorphous alloy — the beginning of the still-expanding field of metallic glasses.

HL: Why are they called metallic glasses?

PD: This is a very logical name. The definition of a glass is a solid obtained from a liquid which, when cooled, does not crystallize. This is exactly what we obtained. Now there are international meetings on metallic glasses and books on metallic glasses.

We continued to look for new alloys, the first one being a magnetic alloy. Can we get ferromagnetic behavior in a solid which is not crystalline? The first thing that comes to anybody's mind if you want something ferromagnetic is to use iron, nickel, or cobalt — the three most important magnetic atoms. Iron was the first choice. So we tried to find something that, mixed with iron, would quench into the amorphous state; and, having an empirical approach as to what to try, we chose phosphorus. A student by the name of Lin was doing the experiment, and he made a mistake. He forgot that it should not be melted in graphite. He had a graphite insert in the high-speed gun, shooting a globule of liquid iron-phosphorus alloy. The iron reacted with graphite, and it formed an alloy which contained also carbon. And this third alloy, iron-phosphorus-carbon, was amorphous and turned out to be one of the relatively strong amorphous ferromagnetic alloys. Iron-phosphorus alone was not quite amorphous. It turned out to be a very lucky mistake.

We continued to be interested in magnetism, of course, but there was something else to look for that could exist also in the amorphous state of metallic glasses. That was superconductivity, which is zero electrical resistance at low temperature, discovered by Kamerlingh Onnes in 1907. It was never considered a promising field in technology because it requires liquid helium, and people thought that it could never be applied on a large scale. Our question was to find alloys to quench. First, we must have a superconducting metal. Second, we must add a metalloid which will make it possible to be amorphous in the ratio of 75/25 or 80/20 (an empirical rule but a good one to follow). So we looked at the superconducting metals such as niobium and, finally, lanthanum, a good candidate for other reasons. Lanthanum-gold was chosen, tested, and turned out to be a superconductor below about 3.5 Kelvin. In looking for promising alloys for practical application, you want the highest possible transition temperature. The maximum reach now is about 18 to 20 K for some alloys — but they are crystalline alloys. For amorphous alloys, the first one we discovered was only 3 or 3.5; but we are up to about 10 K already.

Assuming that we cannot get anything better because it essentially cannot be done with a glassy structure, why do we still carry on research on these alloys? Well, being an educational institution, maybe we should not worry about answering the question from a practical standpoint. It's just science, and we could go on without justifying it except to say that there are new things to explain and many new things to introduce in the theory. But it's a little better if you can find a practical reason, and there is one that I think is very important. The crystalline materials are progressively damaged by high radiation fields; and superconductivity, when the alloy is exposed to high radiation doses (a transition temperature of, say, 18 K for some alloys), goes down to 3 or 4 K. So these amorphous alloys would be useful if the amorphous structure is insensitive to radiation. This has been confirmed recently by experiments made by William Johnson [associate professor of materials science at Caltech] on amorphous superconducting alloys subjected to high doses of radiation in the reactor at Livermore. Currently, there is also quite a bit of excitement on the chemical corrosion resistance of some of these alloys. Some of them are excellent, and they should also be cheaper than ordinary stainless steel.

I must say that what are still behind in metallic glasses are methods of fabrication. In this lab we always were satisfied with very small specimens, maximum one inch in diameter. That's enough for fundamental studies. But if these are going to be applied, we must have sheets of materials that are large enough for transformers or for magnets. And we have some hope that even massive metallic glasses will be obtained in the future. □

The Chromium Mechanism

The first comprehensive explanation of electrochemical activity during the plating of chromium has recently been formulated at the General Motors Research Laboratories. This understanding has aided in transforming chromium plating into a highly efficient, high-speed operation.

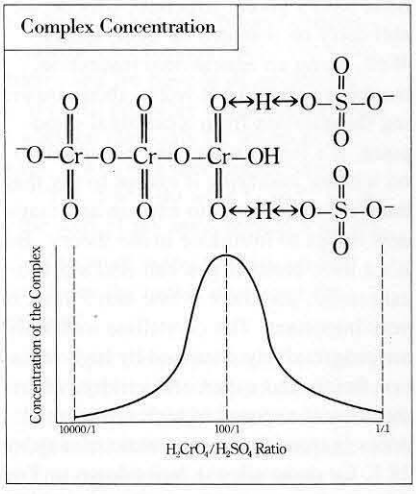


Figure 1: The electroactive complex and a theoretical plot of its concentration as a function of chromic acid to sulfuric acid ratio.

Figure 2: The electroactive complex diffuses from the bulk electrolyte solution (A) through the diffusion layer (B) to the Helmholtz double layer (C) to be discharged as metallic chromium (D) on the cathode (E) surface.

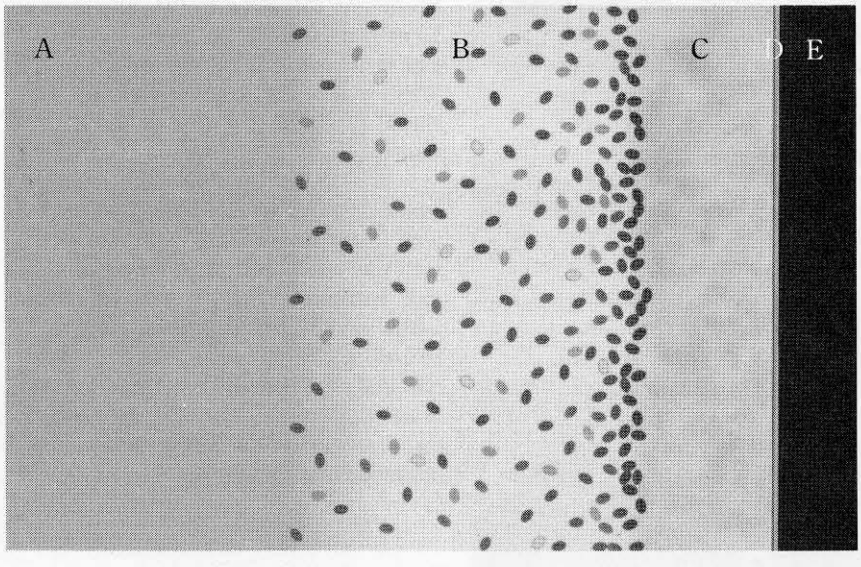
FOR MANY industrial applications, chromium coatings of more than 0.2 mil thickness are required for wear and corrosion resistance. But the conventional method of plating chromium is neither fast nor efficient. Nor, until the recent work of a GM researcher, had the steps involved in the century-old plating process been explained in detail. Through a combination of theory and experiment, Dr. James Hoare has devised the first comprehensive mechanism for chromium plating. This increased understanding has helped electrochemists at the General Motors Research Laboratories develop a system that plates chromium sixty times faster than the conventional method, while improving energy-efficiency by a factor of three.

The electrolyte for plating is a chromic acid solution which contains various chromate ions: chromate, dichromate and trichromate. From a series of steady-state polarization experiments, Dr. Hoare concluded that trichromate is the ion important in chromium deposition.

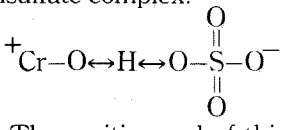
Sulfuric acid has been recognized as essential to chromium plating and has been assumed by some to be a catalyst for the process. In this strongly acidic solution, sulfate should be mostly present as the bisulfate ion $(HSO_4)^-$. Dr. Hoare found, contrary to expectations, that the addition of sulfuric acid to the plating bath decreased the conductivity of the solution.

Combining these findings with the results of previous investigations, Dr. Hoare concluded that the electroactive species was a trichromate-bisulfate complex (see Figure 1). From equilibrium considerations, he theorized that the maximum concentration of this species occurred at a 100-to-1 chromic acid/sulfuric acid ratio. The observation that the maximum rate of chromium deposition also occurred at this ratio supports the conclusion that this trichromate-bisulfate complex is the electroactive species.

During the plating process, the complex diffuses from the bulk solution toward the cathode (see Figure 2). Electron transport takes place by quantum mechanical tunneling through the potential energy barrier of the Helmholtz double layer and the unprotected chromium in the complex (Cr atom



on the left in Figure 1) loses electons by successive steps, going from Cr^{+6} to Cr^{+2}. Decomposition of the resulting chromous dichromate complex takes place by acid hydrolysis to form a chromous-oxybisulfate complex:



The positive end of this complex is adsorbed onto the cathode surface. Electrons are transferred from the cathode to the adsorbed chromium ion, forming metallic chromium and regenerating the $(HSO_4)^-$ ion. Thus, Dr. Hoare's mechanism explains how sulfuric acid, in the form of the bisulfate ion, participates in the plating process.

IT HAS long been known that chromium cannot be plated from a solution when initially present as Cr^{+3} because of the formation of the stable aquo complex, $[Cr(H_2O)_6]^{+3}$. Yet chromium can be plated when initially present as Cr^{+6} even though it must pass through the Cr^{+3} state before being deposited. Dr. Hoare's mechanism handles this paradox by explaining that the chromium ion being deposited (on the left in Figure 1) is protected by the rest of the complex as it passes through the Cr^{+3} state, so that the stable aquo complex cannot form.

The diffusion of the electroactive complex apparently controls the rate of the process, so that shortening the diffusion path increases the speed of chromium deposition. A high rate of relative motion between the electrolyte and the cathode will shorten the path. This can be accomplished by rapid flow or by agitation of the electrolyte.

Dr. Hoare found that the rate of chromium deposition increased with electrolyte flow until the process was no longer diffusion-controlled. He also found that the use of dilute electrolyte significantly increased plating efficiency.

"This project is an excellent example," says Dr. Hoare, "of how basic research and engineering principles can be combined to develop a new, successful process. Now, we'd like to take on the challenge of plating successfully from Cr^{+3}, which would be an even more efficient way to provide corrosion and wear resistance."

THE MAN BEHIND THE WORK

Dr. James Hoare is a Research Fellow at the General Motors Research Laboratories. He is a member of the Electrochemistry Department.

Dr. Hoare served as an electronics technician in the U.S. Navy during the Second World War. In 1949, he received his Ph.D. in physical chemistry from the Catholic University of America. After an assistant professorship at Trinity College in Washington, D.C., he joined the US Naval Research Laboratory as a physical chemist. He became a staff member at General Motors in 1960.

Dr. Hoare's sustaining interest has been in electrochemical kinetics and the mechanisms of electrode processes. He is best known to the scientific community for his basic studies of hydrogen and oxygen electrode mechanisms. His book, *The Electrochemistry of Oxygen,* published in 1968, is considered a work of primary importance to the field. In addition to his work on chromium plating, he is responsible for the fundamental research that helped make electrochemical machining a precision process.

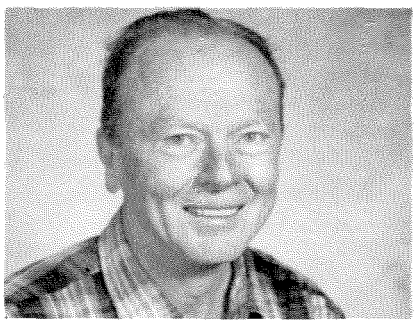
Random Walk

New Provost

ROCHUS E. VOGT will become vice president and provost of the Institute on April 1, replacing Institute Professor of Chemistry John Roberts. Vogt, the first R. Stanton Avery Distinguished Service Professor, is also currently chairman of the Division of Physics, Mathematics and Astronomy. He came to Caltech in 1962 after receiving his SM and PhD degrees from the University of Chicago.

Vogt is an authority in research on cosmic rays and co-founder with physicist Edward Stone of the Caltech Space Radiation Laboratory, whose new instruments and experiments have led to significant advances in understanding of these rays. He is currently principal investigator on the Voyager 1 and 2 cosmic ray experiments. He is the author of numerous scientific papers in the field of high-energy astrophysics, a fellow of the American Physical Society, and a recipient of a NASA Exceptional Scientific Achievement Award.

Driver Education



PROBABLY drivers of automobiles thrusting down the highways and byways of the world seldom wonder why they are going down the right — or the left — side of the road. But alumnus Richard Hopper (PhD '39) has used his all-over-the-world travels as an oil geologist for some 40 years, first, to observe the varying customs, then to do some research into their origins, and, finally, to write an article about what he found out. "Left-Right: Why Driving Rules Differ" was recently published in *Oil Progress*, a publication of Caltex Petroleum Corporation that is not distributed in the United States; an adaptation of the article appeared in the October 1982 *Transportation Quarterly*. Hopper deduced that the reasons some countries drive on the right and others on the left derive from the following facts:

- About 90 percent of the human race is, and always has been, right-handed.
- In the Middle Ages this right-handedness motivated travelers, on foot or horse, to keep to the left for defensive purposes in passing oncoming traffic, so as to be able to use swords or lances in right hands. In recognition of this custom Pope Boniface VIII in 1300 A.D. declared that "all roads lead to Rome," and directed pilgrims to keep to the left side of the road. This edict had something of the force of law in much of western Europe for 500 years.
- Beginning about 1750, however, in America and France large freight wagons were built Conestoga-style, with no drivers' seats but with the driver astride the left rear horse of the six- or eight-horse team, using his long whip in his right hand. This impelled drivers to edge to the right in passing oncoming wagons, so as to be able to see down to their left and avoid banging axle hubs or wheels when passing closely on narrow roads. The first law in this country making right-hand travel compulsory was a Pennsylvania state law of 1792.
- In Britain, by contrast, freight wagons were smaller and built with drivers' seats at the front. On these seats the drivers naturally sat as far to the right as possible, so as to use their long whips in their right hands without interference from the loads behind them. This motivated drivers to edge to the left in passing oncoming vehicles, again so as to be able to see down and pass closely without hitting. Britain's first law requiring keep-left traffic was passed in 1756.
- Today, three of the world's five most populous countries — the USA, the USSR, and China — use the right side. The other two — India and Indonesia — use the left. Most countries now or once under British rule still keep to the left; exceptions are the USA, Canada, Nigeria, Burma, Belize, and Gibraltar.
- The Swedish were the last of the mainland Europeans to drive on the left, and they switched to the right in 1967. A more recent change took place in Okinawa, where residents drove on the right during the American occupation after World War II. But in 1978, after again becoming part of left-driving Japan, the island switched back to the left. The total cost of the change, made over an eight-hour-long, no-traffic-allowed period, is estimated at $80 million.

Hopper retired in 1979 from his position as vice president of American Overseas Petroleum Limited in New York City; he now lives in Connecticut.

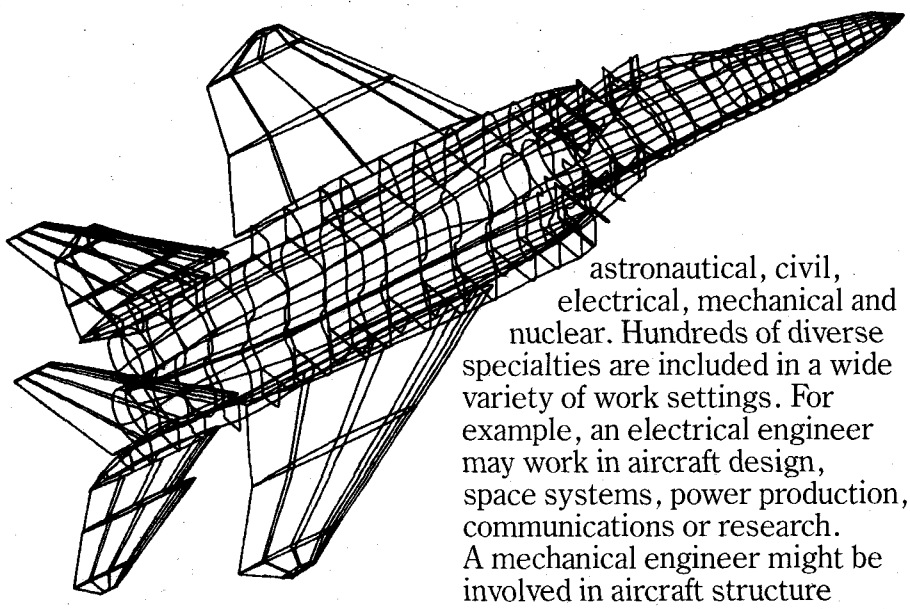
Richter Seismo Lab

Back in 1932, Charles Richter (now professor of seismology emeritus at Caltech, devised a quantitative way of describing the magnitude of an earthquake — the well-known Richter Scale. In honor of this achievement and many others, the Charles F. Richter Seismological Laboratory was recently dedicated at UC Santa Cruz, and Richter himself was happily there in person.

In Memoriam

BRUCE SAGE, professor of chemical engineering, emeritus, since 1974, died on January 11 at the age of 73. Sage, who held two Caltech degrees (MS '31, PhD '34), became a research fellow at the Institute in 1934. Just ten years later he was a full professor. His contributions to the study of the physical properties of hydrocarbons resulted in major advances for petroleum technology. Among his awards were the national Medal for Merit for work on the manufacture of propellants during World War II and the Naval Ordnance Test Station's Thompson Award for management of the rocket ordnance program. He is survived by his wife, Helen, of Winston, N.M., and South Laguna.

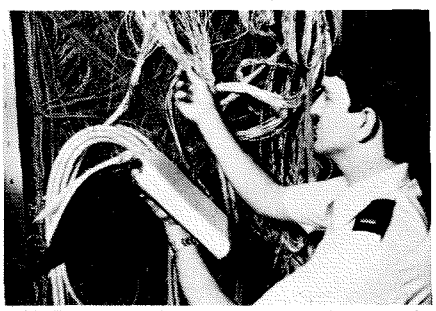
ENGINEERING TAKES ON EXCITING NEW DIMENSIONS IN THE AIR FORCE.



Computer-generated design for investigating structural strengths and weaknesses.

Developing and managing Air Force engineering projects could be the most important, exciting challenge of your life. The projects extend to virtually every engineering frontier.

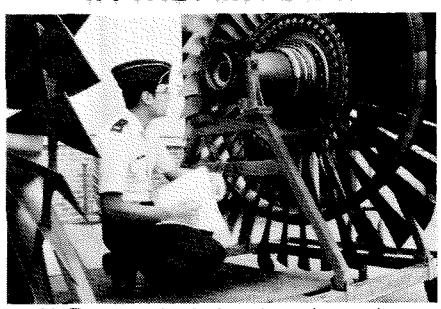
8 CAREER FIELDS FOR ENGINEERS



Air Force electrical engineer studying aircraft electrical power supply system.

Engineering opportunities in the Air Force include these eight career areas: aeronautical, aerospace, architectural, astronautical, civil, electrical, mechanical and nuclear. Hundreds of diverse specialties are included in a wide variety of work settings. For example, an electrical engineer may work in aircraft design, space systems, power production, communications or research. A mechanical engineer might be involved in aircraft structure design, space vehicle launch pad construction, or research.

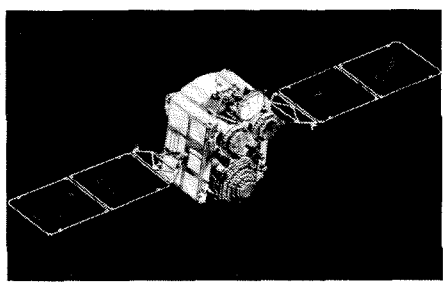
PROJECT RESPONSIBILITY COMES EARLY IN THE AIR FORCE



Air Force mechanical engineer inspecting aircraft jet engine turbine.

Most Air Force engineers have complete project responsibility early in their careers. For example, a first lieutenant directed work on a new airborne electronic system to pinpoint radiating targets. Another engineer tested the jet engines for advanced tanker and cargo aircraft.

OPPORTUNITIES IN THE NEW USAF SPACE COMMAND



Artist's concept of the DSCS III Defense Satellite Communications System satellite. (USAF photo.)

Recently, the Air Force formed a new Space Command. Its role is to pull together space operations and research and development efforts, focusing on the unique technological needs of space systems. This can be your opportunity to join the team that develops superior space systems as the Air Force moves into the twenty-first century.

To learn more about how you can be part of the team, see your Air Force recruiter or call our Engineer Hotline toll free 1-800-531-5826 (in Texas call 1-800-292-5366). There's no obligation.

AIM HIGH AIR FORCE

ALUMNI FLIGHTS ABROAD

This program of tours, originally planned for alumni of Harvard, Yale, Princeton, and M.I.T., is now open to alumni of California Institute of Technology as well as certain other distinguished colleges and universities. Begun in 1965 and now in its sixteenth year, it is designed for educated and intelligent travelers and planned for persons who might normally prefer to travel independently, visiting distant lands and regions where it is advantageous to travel as a group.

The program offers a wide choice of journeys to some of the most interesting and unusual parts of the world, including Japan and the Far East; Central Asia, from the Khyber Pass to the Taj Mahal and the Himalayas of Nepal; the surprising world of South India; the islands of the East, from Java and Sumatra to Borneo and Ceylon; the treasures of ancient Egypt, the world of antiquity in Greece and Asia Minor; East Africa and Islands of the Seychelles; New Guinea; the South Pacific; the Galapagos and South America; and more.

REALMS OF ANTIQUITY: A newly- expanded program of itineraries, ranging from 15 to 35 days, offers an even wider range of the archaeological treasures of classical antiquity in Greece, Asia Minor and the Aegean, as well as the ancient Greek cities on the island of Sicily, the ruins of Carthage and Roman cities of North Africa, and a comprehensive and authoritative survey of the civilization of ancient Egypt, along the Nile Valley from Cairo and Meidum as far as Abu Simbel near the border of the Sudan. This is one of the most complete and far-ranging programs ever offered to the civilizations and cities of the ancient world, including sites such as Aphrodisias, Didyma, Aspendos, Miletus and the Hittite citadel of Hattusas, as well as Athens, Troy, Mycenae, Pergamum, Crete and a host of other cities and islands of classical antiquity. The programs in Egypt offer an unusually comprehensive and perceptive view of the civilization of ancient Egypt and the antiquities of the Nile Valley, and include as well a visit to the collection of Egyptian antiquities in the British Museum in London, with the Rosetta Stone.

SOUTH AMERICA and THE GALAPAGOS: A choice of itineraries of from 12 to 29 days, including a cruise among the islands of the Galapagos, the jungle of the Amazon, the Nazca Lines and the desert of southern Peru, the ancient civilizations of the Andes from Machu Picchu to Tiahuanaco near Lake Titicaca, the great colonial cities of the conquistadores, the futuristic city of Brasilia, Iguassu Falls, the snow-capped peaks of the Andes and other sights of unusual interest.

EAST AFRICA—KENYA, TANZANIA AND THE SEYCHELLES: A distinctive program of 5 outstanding safaris, ranging in length from 16 to 32 days, to the great wilderness areas of Kenya and Tanzania and to the beautiful islands of the Seychelles. The safari programs are carefully planned and comprehensive and are led by experts on East African wildlife, offering an exceptional opportunity to see and photograph the wildlife of Africa.

THE SOUTH PACIFIC and NEW GUINEA: A primitive and beautiful land unfolds in the 22-day EXPEDITION TO NEW GUINEA, a rare glimpse into a vanishing world of Stone Age tribes and customs. Includes the famous Highlands of New Guinea, with Sing Sings and tribal cultures and customs, and an exploration of the remote tribal villages of the Sepik and Karawari Rivers and the vast Sepik Plain, as well as the North Coast at Madang and Wewak and the beautiful volcanic island of New Britain with the Baining Fire Dancers. To the south, the island continent of Australia and the islands of New Zealand are covered by the SOUTH PACIFIC, 28 days, unfolding a world of Maori villages, boiling geysers, fiords and snow-capped mountains, ski plane flights over glacier snows, jet boat rides, sheep ranches, penguins, the Australian "outback," historic convict settlements from the days of Charles Dickens, and the Great Barrier Reef. Optional visits can also be made to other islands of the southern Pacific, such as Fiji and Tahiti.

CENTRAL ASIA and THE HIMALAYAS: An expanded program of three itineraries, from 24 to 29 days, explores north and central India and the romantic world of the Moghul Empire, the interesting and surprising world of south India, the remote mountain kingdom of Nepal, and the untamed Northwest Frontier at Peshawar and the Punjab in Pakistan. Includes the Khyber Pass, towering Moghul forts, intricately sculptured temples, lavish palaces, historic gardens, the teeming banks of the Ganges, holy cities and picturesque villages, and the splendor of the Taj Mahal, as well as tropical lagoons and canals, ancient Portuguese churches, the snow-capped peaks of the Himalayas along the roof of the world, and hotels which once were palaces of maharajas.

THE FAR EAST: Itineraries which offer a penetrating insight into the lands and islands of the East. THE ORIENT, 30 days, surveys the treasures of ancient and modern Japan, with Kyoto, Nara, Ise-Shima, Kamakura, Nikko, the Fuji-Hakone National Park, and Tokyo. Also included are the important cities of Southeast Asia, from Singapore and Hong Kong to the temples of Bangkok and the island of Bali. A different and unusual perspective is offered in BEYOND THE JAVA SEA, 34 days, a journey through the tropics of the Far East from Manila and the island fortress of Corregidor to headhunter villages in the jungle of Borneo, the ancient civilizations of Ceylon, Batak tribal villages in Sumatra, the tropical island of Penang, and ancient temples in Java and Bali.

Prices range from $2,350 to $4,500 from U.S. points of departure. Air travel is on regularly scheduled flights of major airlines, utilizing reduced fares which save up to $600.00 and more over normal fares. Fully descriptive brochures are available, giving itineraries in detail and listing departure dates, hotels, individual tour rates and other information. For full details contact:

ALUMNI FLIGHTS ABROAD
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