

A Renaissance on Hamilton Walk

For the past quarter of a century, biology has been undergoing a revolution; five years ago Penn determined to become a part of it.

According to Penn biologist John J. Cebra, the revolution in the field unfolded in two ways: "One revolution had to do with a jump forward in our general understanding of the molecular level of how life processes work; the other, with a massive embracing of biological processes by companies making products."

The first revolution was the coming together of twenty-five years of research—the evolution of techniques to analyze molecules and grow cells in tissue culture, coupled with a dramatic transfer of information that led to cloning cells and altering genes. Across the nation, biologists were learning new ways of growing cells in tissue culture and using biochemical techniques to examine life processes. In the commercial phase of the revolution, these new methods and techniques are being adapted by companies whose reputations depend on standard products of measurable quality.

During much of this period, Penn's biology faculty, whose traditional strengths were in population genetics, ecology, animal behavior, and neurosciences, was swamped with more and more undergraduates: about 2,500 to 3,000 undergraduates were taking biology, and there were 600 to 800 majors. At the same time, faculty in the health schools and the Wistar Institute were agitating for the University to add biology faculty in the new areas of biochemical and molecular biology.

"The time between the development of a new methodology for studying lower animal forms and its application in understanding physiological states in humans has grown very short," explained Dr. Cebra. "And so in fact the Biology Department acts as a kind of conduit for all of the basic information in the biological sciences to the biomedical areas."

Finally, in 1978, the University decided to act. It committed itself to increasing the biology faculty by 20 percent and to upgrading the department's

physical facilities. Dr. John J. Cebra was recruited from Johns Hopkins to direct the process as the department's chairman.

"I tried to coordinate and organize a program and come up with a case study of what we should develop," explained Dr. Cebra, who noted that people within the department had been pointing out the need for such action for years. Dr. Cebra recruited Dr. Stephen Roth, who has just become chairman and will see the development process through its final phase.

The most visible change in the department is the \$6.3 million renovation of Leidy Labs. This historic landmark, called the most modern biological laboratory of its time by the scientific publications of 1910, had changed little since. Alumni of forty to fifty years could still go back to find their bench from freshman biology lab. While Leidy's historical facade and other outstanding architectural features have been retained, the interior has been renewed with oak and chrome and revitalized with modern systems to better service the needs of contemporary biologists.

There were other changes. The biology curriculum was reorganized, with fewer core courses being required. These core courses now draw on examples from both plant and animal sciences, since biological principles can be illustrated with either. More intermediate level laboratory courses are now offered, enabling faculty members to teach subjects

closer to their own research interests and allowing students the opportunity to explore certain areas in depth. Journal clubs, seminars, and research conferences have been developed for graduate students so they can broaden their knowledge beyond their own specialty.

Observed Dr. Cebra, "There is no doubt that over the last four years, the quality of our incoming students has really gone up in a way that goes against the national trend in which the pool of graduate students is shrinking and most students are going to professional schools."

Most importantly, the department had the opportunity to build up its faculty; some five new positions were created and another nine faculty were either retiring or leaving the University.

Dr. Cebra and his colleagues recruited four new faculty members to work in the area of molecular genetics using recombinant DNA techniques (see page 6). These biologists built on the department's historical strengths in embryogenesis and developmental biology.

New appointments were also made from among scientists who were using new biochemical techniques to study cell development and differentiation. The work of these new faculty members ties in closely with another departmental strength in cell physiology, particularly cell motility or how molecules move within the cell and along the cell surface.

New faculty were added in the department's traditionally strong section



in population genetics and ecology, where research is being conducted on such topics as signal behavior in birds and the mathematical theory of population genetics to understand how evolution is driven by strong selective forces.

The final phase in the department's current development program is the building of the Plant Sciences Institute. "Plant Sciences are largely ignored in biological research," explained Stephen Roth, chairman of the department. "It's very difficult to impress on people how little we know about plants chemically." The Plant Sciences Institute will be housed in a new building between the greenhouse and the biology pond, and will also draw on the resources of the Morris Arboretum.

Plant scientist Dr. Andrew Binns was recently recruited to help develop the Institute and will be joined by two other faculty members next year. Dr. Binns traces our current lack of information about plant biochemistry to a shortage of funds for research in this area over the last several decades. While scientists have been splicing and cloning genes, and using biochemical techniques in animal cells for several years, Dr. Binns and his colleagues are just now applying genetic engineering techniques to plants. He sees the Institute not only as an opportunity to bring together colleagues in a challenging, interdisciplinary environment, but also as an assurance that biology students will be exposed to plant science.

"Industry is clamoring for people," he continued. "All over the country, they are setting up basic and applied research labs in plant sciences."

The reorganization of the curriculum, the new physical facilities, and the arrival of fourteen new biologists have stimulated much new research: Assistant Professor Rocky S. Tuan is growing chick embryos in plastic bags to study bone formation from calcium; Daniel H. Janzen is traveling through a rain forest in Costa Rica to document its ecological systems; Lewis G. Tilney has learned that the molecules on the surface of cells bend over when hit by sound waves enabling us to hear. In all, there are thirty-three members of the faculty, each actively involved in biological research. Following are brief descriptions of some of the exciting projects being undertaken by them.

Stalking Infection

Our body's white cells are destroying bacteria and other foreign objects all the time to prevent us from getting infections. Associate Professor of Biology Sally H. Zigmond is studying how these blood cells find the bacteria to destroy them.

These cells, known as polymorphonuclear leukocytes, make up 70 percent of the white blood cells in our blood stream. "Although we aren't aware of it, these cells are constantly fighting all kinds of diseases that we never get because they have done their job," explained Dr. Zigmond.

Originally made in the bone marrow, polymorphonuclear leukocytes circulate through the blood stream for about twelve hours, then move through the walls of the blood vessels to the tissues—"scavenging for bacteria" as Dr. Zigmond puts it. The question is, how do they find them?

Bacteria or foreign agents release fragments into the body's tissues. Dr. Zigmond and her colleagues have been able to show that polymorphonuclear leukocytes actually sense a difference in the concentration of these fragments, and turn and move in the direction where the concentration of these products is greater.

Using a time-lapse movie camera, Dr. Zigmond was actually able to film the way these cells change their shape from stationary round cells to moving cells with ruffles on their front and tails on their rear. When they sense bacterial fragments, they turn in that direction and continue to move toward higher concentrations of the fragments until they arrive at the site of the infection. They then kill the bacteria by surrounding them and digesting them with enzymes. These white blood cells, moreover, can sense minute differences in the amount of bacterial fragments on either side of them.

This process of moving toward a chemical source, called chemotaxis, was known to be at work in the way salmon return to their place of birth to spawn and bacteria find a food source such as sugar or amino acid. Dr. Zigmond discovered how cells in such higher mammals as humans detect the bacterial

fragments and therefore exhibit chemotaxis. She believes this process may be at work in the way cells know how to move when an embryo is developing and when tumor cells attract the cells necessary to grow their vascular system.

At the moment, Dr. Zigmond and her colleagues have completed work showing exactly how polymorphonuclear leukocytes sense the gradient of bacterial fragments. With an electron microscope, they have actually pinpointed the receptors to which the bacterial fragments attach themselves. To see these receptors, Dr. Zigmond and a graduate student have coupled the tiny peptide, which is a synthetic version of the bacterial fragments, with a larger molecule that is visible through the electron microscope. In this way they have been able to show just where the receptor sites are located. Most, they have found, are located on the front, and only a few are positioned on the tail.

They have also discovered that, rather than sensing a difference in the percentage of receptor sites that are filled, the cell is sensing a numerical difference. It will move just as surely to the right when it has ten sites filled on the right side and two sites filled on the left side as it will when it has 1,000 sites filled on the right and 992 filled on the left.

In an applied research project on this same problem, Dr. Zigmond is working with an engineer to try to develop an assay (test) that will help her understand why some of these leukocytes sometimes seem to be slightly defective in their movement toward bacteria; this could prove to be a factor in some diseases such as periodontal disease. The two are trying to determine whether the difficulty in movement comes about because the cells are not as good at moving or because they are not as good at sensing the differences in concentrations in the bacterial product.

Much of Dr. Zigmond's research is very basic, however. She is now trying to understand the process called pinocytosis—how and why the polymorphonuclear leukocytes internalize certain chemicals and receptors within their membrane.

The Cell's Intelligence

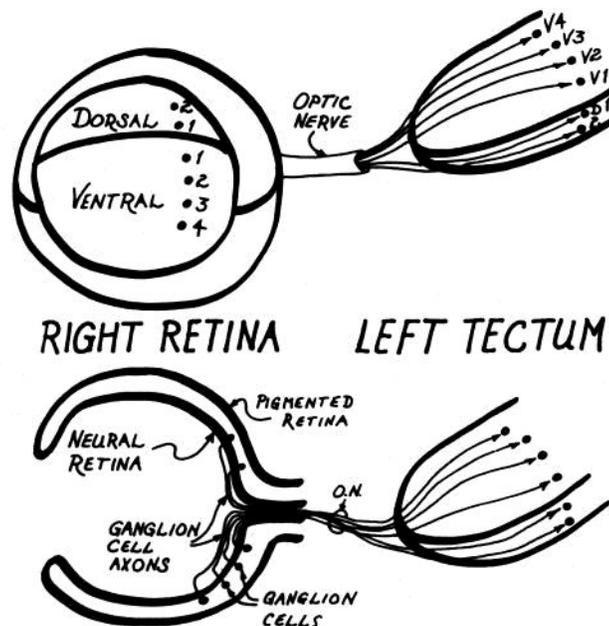
Assume for a moment that cells are automobiles.

"If you're a Martian and you're hovering in a spacecraft over New York City, you would see a mass movement of automobiles going between Scarsdale and New York City in the morning and then back out to the suburbs in the afternoon," said Dr. Stephen Roth, chairman of biology. "You would say, what is going on down there? Is this a random movement, or is it a specific movement? You might pick out a yellow car, mark it, and then try to find out if it always goes to the same place. Well, that is precisely what people in my business do: mark cells to find out if they always go to the same place."

"You would find that out of eight million cars, the marked car will often go to the same place or somewhere very close by, although there will, of course, be exceptions. Immediately you know that you're not dealing with random movement.

"The next question is how do they do it? One possibility is that every single car is on a specific track, and has no choice but to go, say, from Great Neck to the Battery and back. In order to test this theory all you have to do is pick up a car, mark it, and stick it someplace else. When you do this experiment with embryonic cells, you'll find that they will often end up going to the same place they would have before you interfered with them. They'll have to take a different route, but they'll get there. So it doesn't seem to be a track. There is an intelligence in a sense."

The cells, like cars, are responding to markers along the road, and this is what Dr. Roth and his colleagues are studying. The markers, they know, are molecules on the surface of the cells over which the moving embryonic cells are traveling. These cell surface molecules lead the embryonic cells to their normal positions in the process of development. In other words they tell the muscle cell where to grow into long strands, show the nerve cell which way to send out their tendrils



known as axons, and indicate to the bone cells where to start growing into bones. When these surface molecules are not directing the developing cells to the correct destination, birth defects occur.

To study this process Dr. Roth has been trying to pinpoint the cell surface molecules that are the road markers for the optic nerve cells. He chose the optic nerve cells because their route and destination are very clearly established. The cell grows a long tendril, called an axon, from the retina. The axon moves along with other optic nerve cell axons until it gets to a spot in the brain called the tectum where the axons all spread out and go to exactly the same position on the tectum as their other end occupies on the retina. When Dr. Roth manipulates this process with his experiments, the results are clear because the cell either gets to where it is supposed to go or it does not. In most other cells the destination is far less precise.

Dr. Roth and his colleagues have now established that one molecule, called the Tay-Sachs ganglioside, provides a clear cue for the growth of the optic nerve. It is likely to be one of many that lie on the surface of embryonic brain cells and show the optic nerve the direction in which it should develop. It is also the molecule that is not properly processed in Tay-Sachs disease, a hereditary disease that paralyzes and kills predominantly Eastern European Jewish children by the age of four.

Dr. Roth and his colleagues have

been able to show that the Tay-Sachs molecule charts the very specific path that each of the millions of optic nerves must follow to develop correctly in the brain. To get to where they are designed to go, each axon, or extension of the nerve cell, moves out and samples the surface cells around it. When it encounters the right amount of Tay-Sachs ganglioside, it sticks. It then moves a little further to test the surface, and when it finds the next bit of Tay-Sachs ganglioside, it sticks again.

Work is now focusing on trying to identify more of these biochemical cues. It is a painstaking process that involves making the axon radioactive, destroying a certain molecule on the cell surface, and seeing whether the axon still sticks to the cells below.

Along the way Roth and his colleagues have become well-known for developing a technique now used in many different experiments in which cells label those they pass over by leaving behind a radioactive sugar molecule.

Dr. Roth hopes that one day his work will lead him into new fields: "Once we understand the cell surface products that make cells go this way or that way, then we can understand the genes that make them. We can then go back to what Eric Weinberg and Andrew Binns are doing in molecular genetics with very simple gene products and genetically change the shape of organisms."

"Eventually, I hope to be doing molecular genetics as well," concluded Dr. Roth.

A First in the Genetic Engineering of Plants

Pennsylvania biologist Andrew N. Binns and his collaborator Mary-Dell Chilton of Washington University in St. Louis have successfully introduced a new gene into plant cells and regenerated normal plants from them. These plants not only contain the new gene but have passed it along to their own offspring.

In order to accomplish this feat of genetic engineering, the two researchers introduced the new gene into normal plant cells and caused them to grow as a tumor. They then took some of these tumor cells and grew them into normal plants using tissue culture techniques.

The implications of their work are far-reaching:

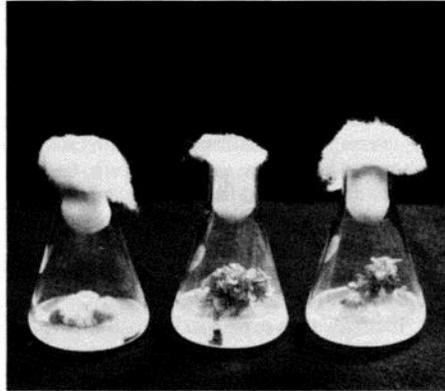
—It is the first time in plants that tumor cells have been consistently altered to grow and produce a normal plant with normal offspring, while retaining the genetic information that caused the tumors. This strongly supports the theory that an organism's normal developmental program can suppress tumor growth.

—This process may well offer scientists the first workable method for genetically engineering plants, opening the way for new lines of research on how each gene works in plants. Such work could eventually improve plants since scientists could alter plant genes to introduce such desirable characteristics as rapid growth, heat resistance, or better bearing capacity.

Prior to the work of Dr. Binns and Dr. Chilton, genes could be introduced into plant cells and plants could be grown in tissue cultures. The problems, however, lay in getting a plant cell with a new gene first to grow and then to incorporate the new gene into its own genetic information in a way that would pass it on to the offspring.

Using tissue culture techniques, Dr. Binns and his colleagues have been working with tobacco plants for five years to clone cells and raise batches of these cells in tissue culture. Drawing on a process that occurs naturally, the researchers infect plants with bacteria containing plasmids that are known to introduce new genes into the plant cells and there-

by cause tumors to grow. They then remove some of the individual tumor cells from the tissue culture and grow each cell into a callus, or ball, of cells in a small glass vial in the laboratory. By making tumors from certain kinds of bacterial plasmids, the researchers can get this ball of cells to grow shoots.



Recombinant DNA technology, which has been widely used in scientific research for the last several years (see page 6), was also involved. Dr. Chilton and her colleagues in St. Louis used recombinant DNA to introduce new genes into the bacterial plasmid's transfer DNA, the part of the DNA of the bacterial plasmid that is incorporated into the plant cell.

Before achieving their success, the scientists had to resolve two problems. First some tumorous plant cells would grow in tissue culture and would form shoots under certain conditions, but would not form roots. Secondly, plants which had been regenerated from the tumor cells, once grown in tissue culture and grafted onto host plants, would set seed, but the progeny did not usually contain the new genes.

Dr. Chilton's laboratory worked with a bacterial strain known as T37, which induced tumors which would not form roots when plants were regenerated. She and her colleagues added a new gene—alcohol dehydrogenase—at a specific location in the T37 plasmid; they believed that they had altered the transfer DNA of the plasmid in a way that would enable the plant cells to grow

roots. However, when they inoculated tobacco plants with this engineered bacterium, they could not get the transformed cells to grow.

Dr. Binns then used a technique first developed in 1956 by his former professor at The Rockefeller University, Armin C. Braun, whom Dr. Binns credits with much of the theory underpinning the current work.

The hormones that would enable the transformed cells to grow into a tumor were apparently not available at the inoculation site. Dr. Binns took the stem of the tobacco plant, cut a section of it, and turned it upside down—putting the apical (top) part of the stem toward the basal (bottom) part of his culture dish and the basal part of the stem toward the top. The plant's growth hormones were then flowing up instead of down (the way they normally flow). He then put a bit of the engineered bacteria on top of the stem, the hormones flowed up the stem toward the genetically engineered cells, and the plant stem made a callus on top. These transformed cells containing the new genes began to grow.

Next, tumor cells were put into a tissue culture made up of basal medium which contained the nutrients that ordinarily enable tumor cells to grow in the culture. Nothing happened. Dr. Binns and his colleagues then added high levels of cytokinin, one of the two hormones that are responsible for plant cell growth. The transformed cells began growing rapidly.

"We got complete plants, which contained this new gene that was introduced to the T1 (Tumor inducing) plasmid, and then we let it set seed, and that gene is being passed on now to the progeny," asserted Dr. Binns.

While this new technique may alter the course of plant science and the nature of plants themselves, Dr. Binns is most interested in it as a technical advance that will help him study how plants grow. For example, how do specific genes on transfer DNA cause the chemical reactions that manufacture the hormones auxin and cytokinin, thereby stimulating plant cell growth?

As Dr. Binns notes, "The exciting part is yet to come. We can now take genes from a plant, put them into a vector (the bacterial plasmid), put them back into a plant, and study how they function. Through this work we may learn how plant genes function in controlling plant growth."

Why Muscles Move

Whether we are playing tennis or wrinkling our foreheads, reading a book, eating a steak, or typing a paper, we are using muscles. How do these muscles move?

That question has been plaguing Lee D. Peachey, professor of biology for the last twenty-five years, since he first looked at the legs of a cockroach under an electron microscope.

A biologist by way of an undergraduate major in engineering and physics, Dr. Peachey became intrigued as a graduate student at the University of Rochester with the new electron microscope and its possibilities. After a brief stay at Rochester, he went to work with Dr. Keith R. Porter at The Rockefeller Institute in one of the few labs then using this new tool.

"This was the time when you could look at anything in the world and it was new and exciting, because we had this tremendously powerful new microscope," he recalled. Dr. Peachey focused on muscle cells.

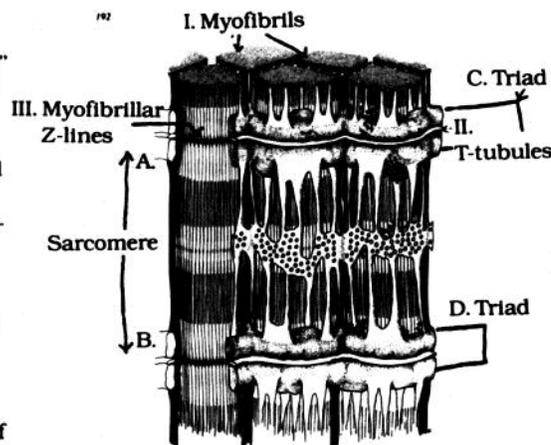
"I look at muscle cell as a cell that has taken one activity—movement—and developed it to an almost absurd level," explained Dr. Peachey. "A muscle produces big mechanical and electrical signals, which makes it easy and fun to study."

It was known that a muscle is made up of tens of thousands of fibers, each one less than a tenth of a millimeter in diameter. Each muscle cell in turn is composed of many myofibrils, bundles of microscopic little strands bound together by a series of membranes. These internal membranes, called the sarcoplasmic reticulum, caught Dr. Peachey's attention, for they were just becoming visible in the new microscope.

Shortly after arriving at The Rockefeller Institute, Dr. Peachey travelled to Cambridge, England with Dr. Porter to visit scientists who had discovered that they could cause just a small part of a live muscle cell to contract by changing the electrical charge at special points on the outside of the muscle cell. However, it would not contract if they stimulated the cell elsewhere. Dr. Porter then showed them pictures of the muscle cell taken

with the use of an electron microscope. It occurred to everyone that it was probably the internal membranes, the sarcoplasmic reticulum, which were connected to the surface in specific places, that was causing the muscle to move.

Dr. Peachey began to document this hunch. "I worked out in my thesis the structure of the membranes in a variety of organisms," he explained. By comparing the frog, the crab, and the lizard, Dr. Peachey was able to define the essentials of the structure. He also realized that fast muscles, those that could contract quickly, had many more of these internal membranes or were very small, while slow muscles had very few of these membranes. This further linked the internal membranes to muscle movement.



Artist's reconstruction of the transverse tubules and sarcoplasmic reticulum surrounding a few myofibrils of a frog twitch skeletal muscle fiber. The myofibrils are located at the top of the illustration (I). One sarcomere occupies the center of the figure (A — B). Horizontal T-tubules (II) form the center elements at triads (C & D) opposite the myofibrillar Z-lines (III). The lateral elements of the triad, above and below the T-tubules are dilated terminal cisternae of the sarcoplasmic reticulum, which are connected across the middle of the sarcomere by a network of tubules and flattened cisternae. (Reproduced, with permission, from the *Journal of Cell Biology*; Peachey, 1965).

He then began to see that one type of membrane crossed through the muscle cell to form a network perpendicular to the strands of the myofibrils. In insects and crustaceans this network looked like a series of tubules, which he and his colleagues called T-tubules. These, they learned, were the part of the membrane that came in from the surface of the muscle cell—a likely conduit for the electrical disturbance (known as the action potential), that caused the cell to move. Penn biologist Clara Franzini-Armstrong first saw the connections of the T-tubules to the surface of muscle fibers in a vertebrate. Dr. Peachey and others were able to extend these studies to other vertebrates by using stains which they put around the cell and later found within the cell.

Dr. Peachey then joined other collaborators for studies on the electrical properties of the system. While they were unable to demonstrate directly that the action potential actually went in through the T-system, they showed that the electrical properties of the T-tubules are in fact very similar to those of the cell's surface membrane where the action potential is known to travel.

"This solved the dilemma, that had existed since the '40s, of how an action potential on the surface of the fiber can cause a contraction in the middle of the fiber within a few milliseconds," summarized Dr. Peachey.

The lure of new technology and an interest in seeing this T-tubule system more clearly led Dr. Peachey to begin work with high voltage electron microscopy. This new high voltage tool enabled Dr. Peachey and his colleagues to use sections that were about ten times thicker than before and afforded them their first look at large regions of the T-system, rather than only little dots of T-tubules. They discovered that the T-tubules were not only connected horizontally, but also longitudinally—by long verticle tubules and helicoids, which work like ramps that connect the floors of parking garages.

Dr. Peachey is now returning to the issue of muscle movement and is currently building a special optical microscope that will permit him to make very accurate studies of the actual muscle contraction itself.

"I like to see how things work," he confesses.

Recombinant DNA: The Stuff of a Revolution in Biology

The ability to link DNA from different organisms into an easily duplicated unit—a process called recombinant DNA—has revolutionized biology.

It has made possible the isolation and purification of genes and the production of large amounts of specific proteins, and has enabled scientists to exchange genetic information from one organism to another.

Scientists can now study the molecular bases of disease, evolution, and embryology and can manipulate genes to remove or add a piece which can, in turn, improve the proteins that the gene is coding the cell to produce.

Recombinant DNA technology permits companies to manufacture antibodies, hormones, and enzymes more efficiently and on a scale, and with a degree of quality, that has thus far been impossible.

It will enable bioengineering firms to manufacture large quantities of substances, like the virus inhibitor interferon, that are made naturally in such small quantity that it is very difficult to get enough of them.

What is this technology, and how are biologists using it at Penn?

What is Recombinant DNA?

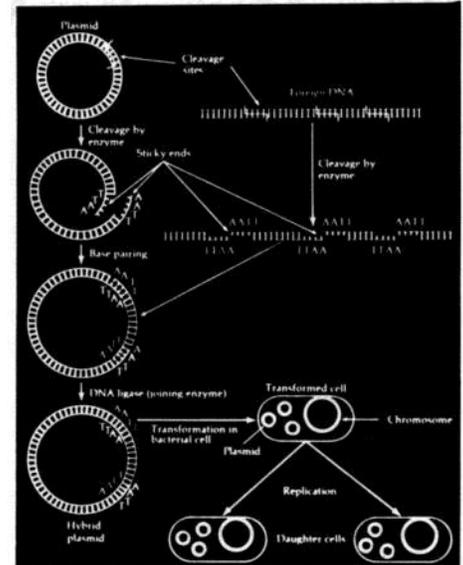
"Basically, recombinant DNA involves taking DNA from one organism and fusing it with DNA from another organism; the combined product is then grown within rapidly dividing cells," explained Dr. Eric S. Weinberg, associate professor of biology, and one of the first in the country to use recombinant DNA techniques.

A cell's DNA is the long two-stranded spiral that contains the organism's genetic information. The DNA is composed of two lines of millions of the same four molecules called bases. The DNA makes RNA, short single-strand replica sequences that contain the information from the DNA which is necessary for the RNA to act as a template for making one of the cell's proteins. These proteins are used as enzymes or structural molecules for the cell.

To develop large quantities of a particular gene, Dr. Weinberg and other scientists must first separate the DNA of one particular gene from the rest of the DNA. To do this they take advantage of the new tools of molecular genetics. Especially valuable is a group of enzymes called restriction enzymes, which cut the DNA like a pair of scissors each time they come across the particular sequence of bases that they recognize—the sequence AATT in the illustration above. The piece of DNA from the organism under study—the cell of a human, a sea urchin, or other organism—is then combined with the DNA of a bacterial plasmid, a little ring of DNA that is found in bacteria (not the bacteria's chromosome, but an extra piece of DNA). When the restriction enzyme cuts the DNA in each of these organisms, it leaves an end of four bases hanging loose as is shown in the illustration. This is called a sticky end. Drops of the DNA fragments from the organism being studied, plasmid DNA, and an enzyme called ligase are incubated together. The sticky ends recognize each other and the ligase joins them together. The plasmid containing the new gene is then added to a suspension of bacteria so that each plasmid enters a particular bacterial cell where it begins to replicate. Single bacterial cells when distributed over agar in a petri dish will grow into colonies containing millions of identical bacteria. A colony is really a clone of the original single cell.

The colony containing just the DNA of interest is distinguished from colonies carrying recombined plasmids with other pieces of DNA. This is done with techniques that expose samples of each colony to radioactive RNA made from the gene that the scientists are seeking. The RNA combines with only the plasmid DNA that contains this gene and reveals itself as a spot on X-ray film and shows the biologists where to find the colony with the gene of interest.

The scientists pick off the colony of bacteria that contains the gene and grow this colony until they have millions of the bacteria containing the plasmid DNA of



the gene they need. They break open the cells and spin them in a centrifuge to separate the chromosomal DNA from the plasmid DNA. Using the restriction enzyme, they cut the plasmid DNA back into the original plasmid piece and the inserted piece from the organism under study. The inserted piece, a particular gene sequence, is then separated from the plasmid DNA. Thus milligrams of the pure DNA can be prepared.

How is the Technology Used at Penn?

Scientists can use these purified genes in many ways. They can make the gene a chemical probe by altering the base sequence and putting it into the cell to see what changes occur in the process they are studying. They can also use it to measure RNA by making it radioactive and seeing how much sticks to the cell's RNA. In short, they use it as a tool with which they can recreate what is occurring in the cells and find out what makes genes work in one cell and not in another or what turns the cell off and on.

Dr. Eric Weinberg is using this tool to study genetic coding for histones—the important proteins that form the structural backbone of the strands of DNA.

"I am working on how one particular gene set—the histone genes—functions in a cell, particularly during the various stages of embryo development. I hope to use the histone genes as a model to understand how the genes turn on and off, as well as to learn about the structure

The Secret of Mother's Milk

While everyone from poets to La Lèche League has proclaimed the virtues of mother's milk, it was Professor John Cebra and his colleagues who were able to show us one of the reasons why.

of genes, and to understand their evolution," explained Dr. Weinberg.

Histone genes, he has learned, come in sets of five, each lying next to the other on the DNA and containing about 6,500 bases for the group. Many of these bases do not actually code for proteins. They are spacers between the genes and interest Dr. Weinberg because they contain regions which are important in turning the genes on and off in some way and other regions which have changed extensively from species to species and are therefore rapidly evolving.

Dr. Weinberg has recently completed a series of experiments to pinpoint just when the histone producing RNA is made in the developing embryo. By measuring the RNA with DNA probes, he has found that histone genes make about ten percent of all the RNA in the embryo from the beginning of the embryo until the blastula stage (when the embryo forms into a hollow sphere). This production tapers off as the cell reaches the blastula stage, and then a second set of histone genes gets turned on. This gene switch is one of the most dramatic rapid changes in gene expression known in animal cells.

Others at Penn use the technique for very different purposes. Dr. Gregory M. Guild is using recombinant DNA technology to study how groups of genes respond to a single signal during development of an organism. He is using the hormone induction of genes in the fruit fly, *Drosophila*, as a model system. Kelly Tatchell is using it to study the control of yeast genes and has succeeded in putting altered genes back into the yeast and testing their function in growing organisms.

Dr. Cecilia W. Lo is experimenting with genes injected into mouse embryos to see what DNA sequences are involved in regulating these genes. Neville Kallenbach is analyzing particular mutations called frameshifts by creating special plasmid DNA changes and studying how mutagens further alter the DNA. And Andrew Binns is studying how particular plasmid genes create normal plants from tumor cells (see page 4). Scientists are only just beginning to explore the uses of recombinant DNA technology in their own research. The results of their work will undoubtedly continue the biological revolution now under way.

Scientists know that mother's milk is full of antibodies which line the infant's stomach and respiratory tract and protect the infant from the antigens (foreign substances) that are constantly coming into its gut, respiratory tract, and lungs. Dr. Cebra and his colleagues were able to demonstrate how a mother's antibodies pass into her milk. Before doing so, however, they had to work out the details of how those antibodies are made in the adult system.

Antibodies are produced by white blood cells known as B lymphocytes which are made in the bone marrow. It was known that some of these lymphocytes end up lining the entire gut wall, the respiratory tract, and the lungs of humans. They lie just under the surface membrane and release their antibodies into the mucus where they then neutralize the antigens and begin the process of destroying them.

Dr. Cebra and his team were able to demonstrate that B lymphocytes travel from the bone marrow to a part of the lymphatic system that lies along the small intestine and the upper bronchi. At about eight to ten sites in the small intestine, there are raised, dome-shaped, bald spots, known as Peyer's patches, filled with millions of lymphocytes. Depending on their genetic information, each lymphocyte is programmed to produce one of over a million different antibodies. In the Peyer's patches, special cells are constantly sampling the antigens that are moving along the gut. Once they identify the antigens in the gut, these cells signal the lymphocytes that produce the proper antibody to begin to divide. It is this ability to sample the environment and reproduce cells with the necessary antibody that protects us continually against new antigens when we go to a different place, change our diet, or in some other way expose ourselves to new foreign substances.

Once the right lymphocytes begin to divide, many of them move out—leaving

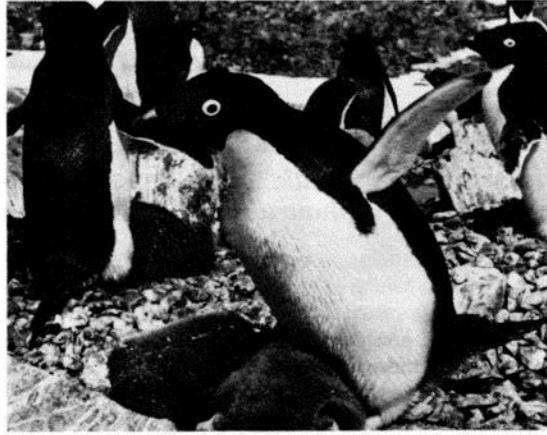
the lymphoid tissues along the small intestine and filtering their way through the lymphatic system to the jugular vein and then into the blood stream. A similar process is occurring in the tissues near the bronchi. In the blood stream they are carried along until they reach the gut, the respiratory tract, and the lungs. Once they reach their destinations, they lodge in the epithelium, or surface membrane, and begin secreting huge amounts of antibody all along the length and breadth of the gut, respiratory tract, and lungs. In four days they are dead. Thus the body has to make these cells continually.

Dr. Cebra and his colleagues then went on to show that B lymphocytes are also at work in mammary tissues. In fact they work in two ways. First, in the early stages of lactation, these cells enter the mammary tissues in the same way that they enter the intestines and lungs—by leaving the blood stream and lodging in the tissue. There they begin to release their antibodies, which then seep through the mammary tissue into the milk and go on to line the infant's gut and respiratory tract: since the infant and mother are sharing the same environment, the child benefits from the same antibodies that the mother's body has been signaled to produce.

The second way that these cells work is to lodge themselves in the mammary tissue and develop cell manufacturing centers right in the mammary glands. In these local manufacturing centers, the cells begin to divide, and large colonies of cells grow on the spot with the specialized B lymphocytes. The cells then secrete their antibodies, and the antibodies work their way directly into the milk. Thus nursing mothers are producing B lymphocytes in their mammary glands as well as in their gut and respiratory tract.

"This is why Similac and all the infant feeding formulas, no matter what Nestle and Ross Labs and Abbott say, are nowhere near as beneficial as mother's milk," concluded Dr. Cebra.

Reports from Antarctica



It is unlikely that Dr. Robert E. Ricklefs complained about the difficulty of performing experiments in the old Leidy Labs. After all, this ecologist, whose research focus on the life history patterns of birds, has traveled to such remote areas as Antarctica and South Georgia Island near the Falkland Islands to conduct his research.

"The area is really spectacular, but it's not comfortable," he observed in describing his work in Antarctica in 1981 and South Georgia in 1982 where he went to learn about the food and energy requirements and reproductive habits of birds in cold temperatures.

"When you're working outdoors hour after hour, and it's cold, and it's wet, and the wind is blowing 30 knots, it's pretty miserable." But, he noted, "the area is pristine and completely remote. And the wildlife is fantastic!"

Dr. Ricklefs has traveled to these extremes to observe birds with their eggs and chicks. He wants to find out how much time different birds spend sitting on their eggs and brooding their young chicks, how much time they spend feeding, and how parents divide up the responsibility. He is also taking many measurements—how much do the chicks grow in what period of time, how much food do the adults bring back for the chicks, what are the energy requirements of the adults, and what are the components of their food?

Once the data are gathered, Dr. Ricklefs hopes that he will be able to see the trade-offs that birds make, particularly in the area of reproduction. If, for example, a bird has to spend all of its time at the nesting site, it has less time for gathering food and less energy for more reproduction. He is particularly

interested in knowing how life history patterns evolve and how they differ among cold temperature birds, such as the auklets and petrels, as well as how their patterns may differ from temperate and warm temperature birds such as the starlings of Pennsylvania or tropical birds of the Pacific islands.

Getting these observations and measurements, however, is not always easy. When Ricklefs went to South Georgia, it was necessary to fly to South America—to Punta Arenas in Chile—then get on a research vessel, in this case a British Antarctic Survey vessel, for a two-week trip out to the island. "All the material that's going from the ship to the island has to be ferried in rubber zodiac-like boats. And it's rough weather. A lot of the equipment had to be sent to England three or four months ahead of time to be shipped down in an earlier supply boat."

All this work is not without its rewards however. Since few are willing to go to such lengths, Dr. Ricklefs explained, "We've been some of the first people to get measurements on these sea birds."

Unlike their counterparts in other parts of the world, the cold temperature birds are not afraid of people. Thus scientists are able to watch them for hours, pick up their chicks and weigh them, or take them back to the lab for blood samples.

"The best information we'll have is for these cold temperature sea birds," observed Dr. Ricklefs.

Dr. Ricklefs has witnessed some dramatic differences in life history patterns. The cormorant chicks, for example, are extremely dependent on the adult and need to be brooded continually for several weeks. Others, such as the skua (a relative of the gull), are so well-insulated

and have such a large heat-generating capacity that even at almost freezing temperatures, they are independent of their parents.

While Dr. Ricklefs has been working in Antarctica and with a student on South Georgia Island, some of his other students are measuring and observing birds in New Brunswick, Alaska, and the Midway Islands in the Pacific as well as in eastern Pennsylvania.

"It turns out that the birds in the Antarctic aren't that much different from those in temperate and warm climates in terms of their reproductive adaptations," he said. "The type of development which birds undergo depends on the taxonomic group they belong to rather than what environment they're found in. Whether a bird nests in the arctic or the tropics, it is going to make some kind of adjustment in terms of the amount of parental care it can give its chicks but that is what differs, not the developmental quality of the chick itself."

Ricklefs is also concerned with how these data fit into the larger issues of life history patterns and their evolution in different species.

"The primary goal is to understand the relationship between life history patterns, so that we can provide realistic models of how these patterns interact in an evolutionary sense," he explained.

What he would like to do eventually is build a computer model which would essentially mimic the evolutionary behavior of some population under different environmental factors so that researchers could interpret the variety of adaptation that is seen in nature in terms of the behavior of the model.

"We want to know which environmental factors have been the most important in producing an evolutionary response. Is it predation? Is it food limitation? Is it variability in certain environments—in temperatures, in breeding areas?" It is research such as his which will provide the data needed to help explain evolutionary differences in species.

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Editors Linda Koons
Michele Steege
