The Cloning of an Antibody

The explosion onto the scene of a technique come of age can be even more dramatic than the ascendance of a new scientific theory. The excitement surrounding development of recombinant DNA methods, for instance, even though dampened by safety concerns, still dominates molecular biology meetings. And the development of Ames's bacterial test for compounds that may cause cancer has triggered a stampede of chemical testing.

Now come the monoclonal antibodies. This technique, as yet unheralded with public fanfare, promises to be as important to research as are recombinant DNA and mutagen detection. Scientists from a wide variety of disciplines are eagerly revising their research plans with the monoclonal antibody picture in mind, and monoclonal antibodies are all the talk at meetings of developmental biologists, virologists, neuroscientists and, especially, immunologists.

Basically, monoclonal antibodies are very specific and very pure biological labels that can be produced in huge (to a cell biologist) quantities and then put to work in a surprisingly large number of ways. The trick in producing them is to "immortalize" cells that produce a particular antibody by merging them with tumor cells. All descendants (the monoclone), which can be grown in a laboratory culture, produce the original, single antibody.

Cesar Milstein, in an interview in his small, crowded office at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England, described how he and George Köhler developed the first successful technique for producing a monoclonal antibody. They were addressing a basic problem, how the two parts of an antibody molecule become joined. "We were working on very esoteric questions," Milstein says. "Only later we realized the [general] potential of the procedure."

Milstein and Köhler immortalized mouse spleen cells that make a variety of desired antibodies. The procedure involved fusing those cells with laboratory grown myeloma cells, a line developed by Michael Potter at the National Institutes of Health from an antibody-producing tumor.

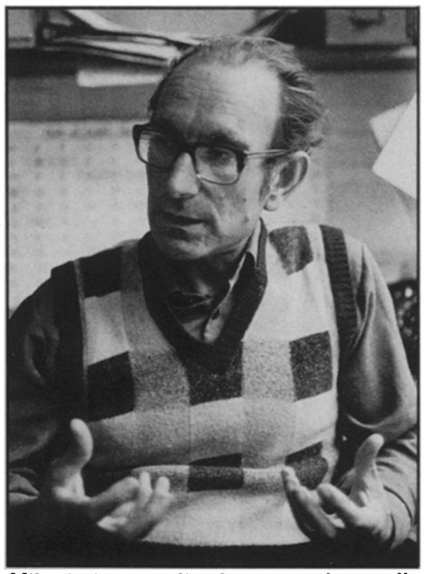
The natural function of an antibody in an animal is to clamp onto a foreign molecule, virus or cell and inactivate the invader. Each species of antibody recognizes one portion, or determinant, of a molecule out of hundreds of possibilities. Rarely does the same determinant appear on different compounds. Once a cell is "committed" to producing a particular antibody, all its descendants make the same one.

Antibodies have long been used as

The ability to produce single antibodies opens new horizons in identifying and purifying rare biological components

BY JULIE ANN MILLER

crude tools in medical diagnosis, such as determining blood types, and in biological experimentation. But their usefulness has been limited by their complexity. If an animal is injected with a pure substance, for instance, it will respond with numerous species of antibodies specific to different sections of the injected molecule. Milstein gestures, showing how an invading right hand could be attacked by different left-hand antibodies recognizing and attaching to any finger or the thumb. The blood serum of the injected animal will



Milstein immortalized mouse spleen cells.

then contain a mixture of antibodies, and that mix will differ from animal to animal or even from time to time in one animal. "It's incredibly complicated," Milstein says. "They [sera] are all horrible as a chemical reagent. If you bleed one animal, that mixture almost certainly will never be reproduced again."

One clone, one antibody

Milstein's new technique has completely solved that serious problem. Each monoclonal antibody is a single chemical entity, one protein with an amino acid sequence that can be determined. And often the cells that produce the antibody can be permanently maintained. The first monoclonal antibody producers are still in good condition in the deep-freeze after four years, Milstein says.

Quantity, in addition to purity, is the reward of monoclonal antibody procedure. Milstein estimates that cells in tissue culture devote at least a third of their total energy to making and secreting antibody. In a liquid medium they produce approximately 50 milligrams of antibody per liter, a concentration similar to weak blood serum. But the volume of cell medium is limited much less than the volume of blood a researcher can draw from an animal. A second technique puts the cells into a mouse and allows them to grow into a tumor. That tumor then makes a huge amount of antibody, up to 20 grams per liter of fluid exuded. "It's easy to make 95 percent pure antibody," Milstein says. "It's sort of a dream of immunologists."

The value of the technique goes beyond production of a pure antibody to a pure antigen (the general term for an antibody's target molecule). The hybrid cells give a pure antibody even in response to so complex a stimulus as a whole virus or a whole cell. When a virus or cell is injected into an animal, the natural reaction is a plethora of different antibodies. But if the hybrid cells derived from fusion of antibody producers and myeloma cells are separated and cultured, the descendants of each hybrid cell produce the same antibody. And that antibody can then be used to purify the random viral or cell component that it binds.

It is as if, to study a forest, botanists had to reach into a grab-bag of unrecognizable seeds, grow the chosen seed and, if the plant is of interest, study it. Eventually one would hope to discover all the plants in the forest.

Among the microscopic "forests" now opening to molecular biologists are the viruses responsible for human disease and the surface components of normal and diseased cells. "Many antigens are lurking on cell surfaces awaiting identification," said Timothy Springer of Harvard Medical School at a recent workshop at the NIH. "The molecules found so far are only the tip of the iceberg."

Preliminary work in many different research areas shows exciting promise for monoclonal antibody procedures. "The results are spectacular," says Hilary Koprowski of the Wistar Institute in Philadelphia. For example, pure antibodies can detect exact differences between viruses and thus provide valuable information on problems important to epidemiology, such as how disease-causing viruses change their protein coats to slip by the active immune system.

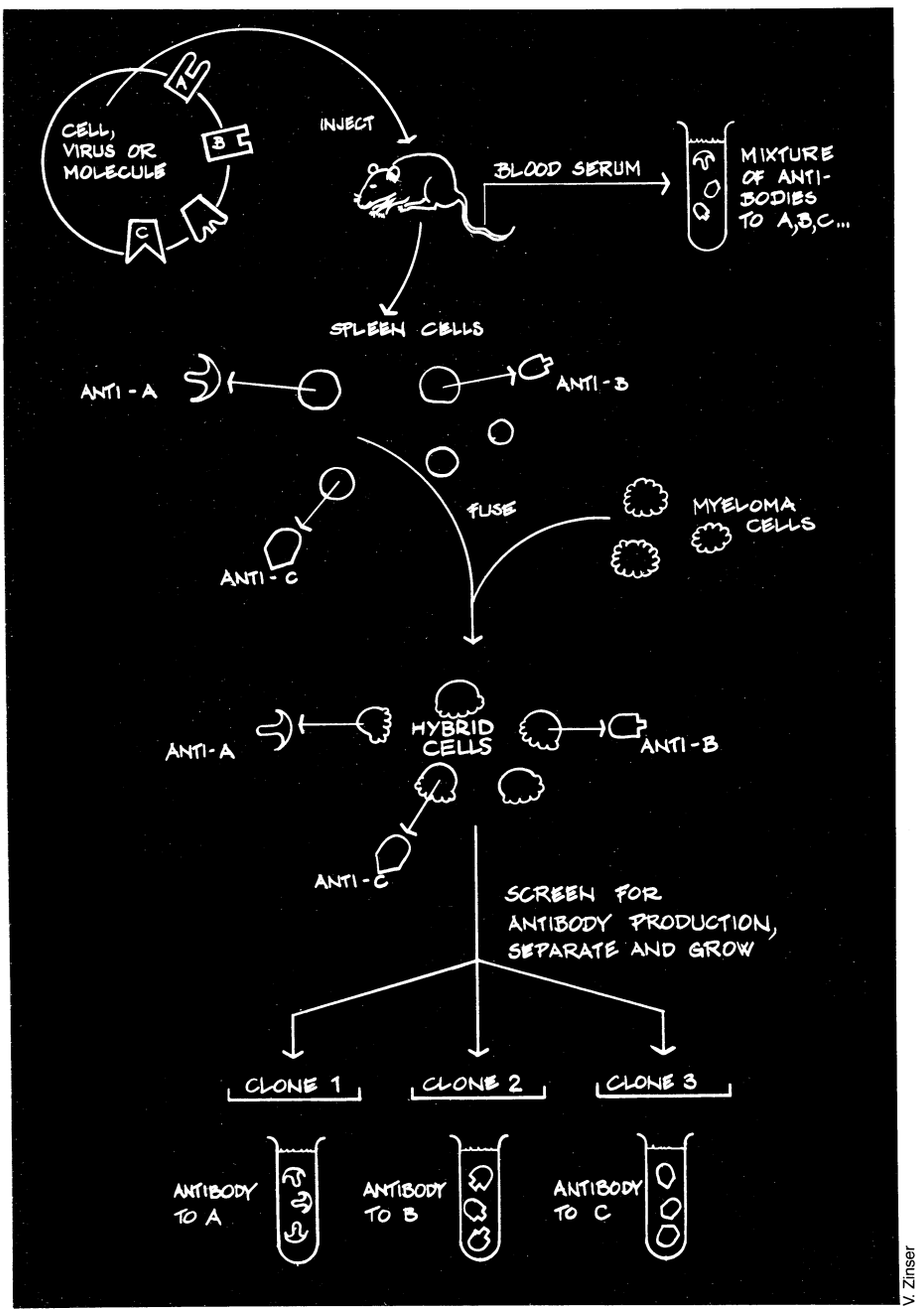
Viruses of many coats

Influenza virus is being examined by Koprowski and Walter Gerhard of the Wistar Institute and Robert G. Webster of St. Jude Children's Research Hospital in Memphis. Their results suggest the changes in coat protein necessary for the virus to start a new epidemic.

The researchers have created a set of 95 monoclonal antibodies that includes antibodies binding to each of at least 55 different viral determinants (they call the antibody groups "clonotypes"). The monoclonal antibodies can reproduce the entire repertoire of antibodies produced by an animal's spleen in response to influenza virus, the researchers find. When they looked at a series of viruses with slight genetic variations, they were able to detect several distinct changes in the set of antibodies that could bind. Yet the heterogeneous collection of antibodies in animal blood serum did not distinguish among the variants and parent.

"This suggests that none of the mutations, although they must have resulted from at least one amino acid substitution, would have been epidemiologically relevant, i.e., none of the variants would have escaped rapid neutralization in a host population with pre-existing immunity to the parental virus," Gerhard and Webster say. It follows that future epidemic virus strains probably must be altered in several amino acids to be successful.

Rabies virus provided a surprising result when analyzed with a set of 10 monoclonal antibodies. Strains of rabies isolated around the world from human, cow, fox, dog and bat had been indistinguishable by standard immunological techniques. But T.J. Wikton and Koprowski demonstrated that monoclonal antibodies that react with one rabies strain may not react at all with another strain. (PNAS: 75, pp. 3938-3942, 1978). Thus the viruses fall into several previously unsuspected groups.



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In response to immunization, an animal produces a mixture of antibodies. However, pure antibodies can be obtained if spleen cells from the animal are fused to cells of a tumor (myeloma) line and the hybrid cells are grown in tissue culture separately.

Inspecting cell surfaces

Understanding the functions of many types of cells found in the body depends on understanding the cell-surface molecules. For instance, the surface molecules of immune system cells are crucial to recognition of and response to foreign material. "The best approach is to identify first cell-surface molecules as antigens (using antibodies), and then to determine their molecular properties," Alan F. Williams and colleagues at the Medical Research Council Cellular Immunology Unit in Oxford say in the December TRENDS IN BIOCHEMICAL SCIENCES.

Because cells differ in their surface components, one use for the monoclonal antibodies is to distinguish cell types. For instance, mature T and B cells of the immune system appear very similar, but their surfaces do react with different antibodies. Another exciting application is to use the antibodies to trace how cells and their surfaces change during development. "The developmental evolution of differentiation antigens [those that vary during development or between different cells] is an important mystery," Milstein says. "It is fundamental to cell biology."

The research results so far have emphasized the diversity of cell-surface components, but investigators are optimistic, rather than discouraged, that they will complete the puzzle. Williams and colleagues say, "The method [monoclonal antibody production] works very efficiently in practice, and, in a relatively short period (years rather than decades), identification could be made of most of the cell-surface molecules of reasonable abundance (say more than 10,000 molecules per cell) on any one cell type."

In one of the earliest studies using monoclonal antibodies, Williams, Giovanni Galfre and Milstein detected five novel membrane components. The researchers had immunized a mouse with immature immune system rat cells and then fused that mouse's spleen cells with myeloma cells (CELL: 12, pp. 663-673, 1977). When five of the resultant clones were examined, all their antibodies were shown to bind specifically to different, previously undefined, antigens on various subpopulations of cells. All five of those antibodies appear to detect minor membrane components;

only 5,000 molecules of one of the antibodies bind per cell. (In contrast, the antibody to a major cell glycoprotein binds 600,000 copies per cell.) The researchers conclude, "The method is therefore extremely sensitive and allows identification down to minor membrane molecules and also of antigens on small subpopulations of a heterogeneous mixture of cells."

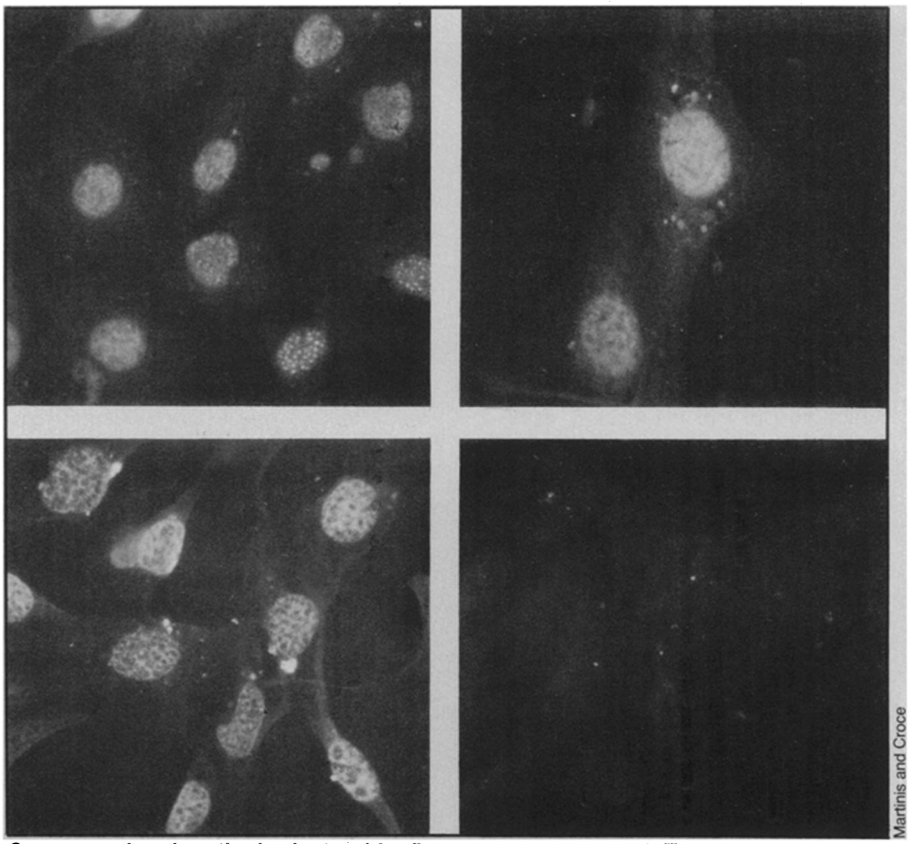
Human cells have been the subject of more recent work, and hybrid cells have been made to produce antibodies of clinical importance. The Cambridge workers, with C. J. Barnstable and W. F. Bodmer of the University of Oxford, injected mice with membranes from human tonsil lymphocytes and made hybrids of myeloma and the mouse spleen cells. One of the six monoclonal antibodies analyzed may be useful in blood typing; it binds to red blood cells of group A_1 and A_2,but not B or O.

In the Nov. 23 NATURE, P. Parham and Bodmer describe a hybrid cell line secreting an antibody that should be useful for tissue typing (for example, for organ transplants). That antibody reacts with the histocompatibility antigen HLA-A_2. Currently, tissue typing antigens are detected by using blood serum from women who have borne several children, but that serum is relatively weak, contains a complex mixture of antibodies and is available only in limited amounts. "In addition to providing tissue typing reagents, these antibodies will be invaluable for the purification, separation and immunochemical analysis of HLA-A, -B, -C, -D molecules," the researchers say.

Development at a membrane

The mysterious biology of development is also being examined with monoclonal antibodies. "This technique is breaking tremendously new ground here," Milstein says. Molecules transiently present on the surface are thought to regulate embryonic cell interactions. Researchers had previously used antibodies from blood serum to follow development, but a single cell component could not be traced definitively or isolated. "A description of the temporal and topographical expression of such an antigen, however, is an important prerequisite to the investigation of any functional role," say Keith R. Willison and Peter L. Stein of the MRC.

The first study of the distribution of a single antigenic determinant in early mouse embryogenesis was reported by Willison and Stern in the August CELL. With colleagues, they immunized a rat with mouse spleen and then fused the rat spleen cells with myeloma cells. The researchers tested the resultant monoclonal antibodies against a variety of tumor cells and chose to investigate an antibody that reacted only against mouse embryonic cancer cells. They found in adult mice that the antibody bound to cells in the spleen, bone marrow, lymph node, brain, kidney and testes, but not to cells in the liver and thymus. That specificity was reminiscent of a component called Forssman antigen, originally described in 1911, during what Milstein calls an "unfashionable" period of immunology. Further analyses by the Cambridge investigators confirmed that the monoclonal antibody binds to a Forssman glycolipid molecule.



One monoclonal antibody, depicted by fluorescence, reacts with T antigen produced in cells infected with virus SV40 (top left) and with virus BK (top right). A different antibody reacts with SV40 T antigen (bottom left) but not with T antigen of BK (bottom right).

The Forssman antigen, as identified by a monoclonal antibody, has an interesting distribution in the embryo. It appears on one region of the early blastocyst, but disappears when the embryo implants. Willison and Stern suspect the molecule plays a role in guiding development, but say that, at the very least, it will be an excellent marker of different subpopulations of cells in a variety of tissues.

A similar result for a different embryonic surface component was obtained in experiments in which a mouse was immunized with embryonic tumor cells, Davor Solter and Barbara B. Knowles of the Wistar Institute report in the November PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES. The antibody they investigated binds a "stage-specific" antigen that appears late in the embryo's 8-cell stage and disappears later. Solter and Knowles conclude they are looking at a glycolipid different from the Forssman antigen.

"We now have the tools to draw developmental maps," Milstein says. "It's like the early days of anatomy when people cut bodies and just looked to see what is inside and how they're connected." In the next few years, he predicts, many researchers will map the normal state of affairs on cell surfaces and then the changes that occur with disease.

Cancer components

Techniques applicable to the study of normal development are usually also applicable to the study of cancer — development gone wrong. Joanne Martinis and Carlo M. Croce of the Wistar Institute are analyzing the surface molecules of cells infected with SV40, a virus that causes cancer in monkeys. SV40 infection has long been known to result in a cell membrane component now called large T (for tumor) antigen and, more recently, in another component called little T. A human virus called BK also produces a similar surface molecule. In their preliminary characterization, Martinis and Croce find that large T and small T react with many of the same monoclonal antibodies, suggesting they are made from overlapping stretches of DNA. In addition, the T antigens from SV40 and BK react with some, but not all, of the same monoclonal antibodies; so the molecules are not identical. Purification of the SV40 T antigen is well underway.

Another approach to malignant cells is to search for more ways in which they differ from normal cells. Koprowski and colleagues have demonstrated for the first time a specific surface molecule on a human tumor. They did it with hybrids of

tumor cells and spleen cells from mice immunized with human skin tumors, called melanomas. They found three categories of monoclonal antibody: some reacted only with melanomas, some reacted with melanomas as well as some other tumors and others reacted with melanomas, some tumors and also normal human cells. "These antibodies suppress growth of melanoma tumors in nude [immune deficient] mice and thus may be directed against a specific tumor antigen," Koprowski explains. "If this is the case, it opens the possibility of 'immunodiagnosis' and eventually immunotherapy of human malignancies." Therefore the monoclonal antibody technique revives an old dream — targeting a destructive element specifically to a tumor.

The monoclonal antibody technique is applicable to research problems ranging beyond cell surfaces. In quite different work, for example, biologists are trying to obtain a monoclonal antibody that will bind interferon, a natural substance that fights viral infections. Monoclonal antibodies could provide a method of purifying large amounts for use in experiments and possibly for clinical applications.

Immortalizing other functions

Going even further, antibody production is not the only transient cell function that can be immortalized in a clone of cells. Perhaps different cells fused with myeloma or other types of tumor cell might provide directly a simple, long-lived source of interferon protein or of other specialized cell products.

Hybrid cell lines have already been established that secrete "suppressor factor," a soluble transmitter chemical thought to regulate the interactions of immune system cells. Ten researchers from England, Australia and the United States, including Leonard A. Herzenberg of Stanford University, recently reported fusing cells of a thymus tumor with appropriate immune system (T) cells, artificially induced to make the factor. The fusion gave rise to a line of cells that secrete suppressor factor. "Such hybrids will be extremely useful in the more refined characterisation of the secreted products of T cells of various types, as unlimited cell numbers can be produced and the titre of suppressor factor produced by the hybrids is much higher than that of conventional factors," the researchers say in the Aug. 3 NATURE.

Other active areas of research already underway include development of monoclonal antibodies that enhance organ transplants, that diagnose and monitor leukemia and that detect subtle changes in the nervous system.

Genes and antibodies

Similarities between monoclonal antibodies and recombinant DNA extend beyond the research excitement each has generated. Workers in the two areas tend to use much the same vocabulary. Both make "hybrids" — either a fused DNA molecule or a fused cell, both "clone" — reproduce that molecule or cell — and both do "shotgun" experiments in which they first hybridize and clone a random assortment of DNA molecules or cells and then select relevant specimens from the then sufficient quantity of material.

Combination of the two techniques promises to engender much semantic confusion, but also a powerful approach to molecular genetics. "One can now contemplate cloning any gene," says Bernard Mach of the University of Geneva. "That was not the case six months ago."

For instance, if a collection of genes is inserted into bacteria and reproduced, the researchers need a way to select among the genes. The most general way would be to look for expression of the inserted gene, but often no measurable activity of the gene product is known. However, if the researcher had monoclonal antibodies to the product of the desired gene, antibodies could identify bacterial cells containing that gene. Mach says, "Give me any antibody directed against a protein and with time that gene can be cloned."

Selling of an antibody

A logistics problem arises as more and more biologists of different talents and training begin incorporating monoclonal antibodies into their research plans. A workshop on cell hybridization in immunology held in November at NIH considered the problem: Does every laboratory need to make its own antibodies or will they be distributed either privately or commercially? Mach predicts a central bank of monoclonal antibodies "either capitalistic or NIH." Koprowski agrees: "We are waiting impatiently for a central depository." The Wistar Institute researchers provide their cell strains to other investigators, but doing so involves much extra work.

Herzenberg says that his laboratory's solution is to provide cells to other laboratories that want to produce large amounts of an antibody, but also to provide the cells to commercial firms that sell antibodies to the "casual" user. (The Cell Distribution Center at the Salk Institute in San Diego stores cells to provide to investigators.) Other researchers, however, claim that companies do not want investigators to be giving away the cells that make monoclonal antibodies the company plans to sell. A major commercial use of monoclonal antibodies will be in kits for radioimmune assays that now contain animal sera.

Milstein explains that none of the basic work on monoclonal antibody production has been patented. Although the technique is now being applied eagerly in laboratories around the world, at the time, he says regretfully, he was unable to convince the MRC administrators that the procedure had important enough applications. □

SCIENCE ON TV

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January 3 (PBS) The Cousteau Odyssey — **"Calypso's Search for the Britannic,"** originally broadcast in 1977, blends myth with documentary in an attempt to solve the mystery of the sinking of the *Britannic*.

January 4 (PBS) NOVA — **"Black Tide"** examines the 1978 *Amoco Cadiz* oil spill disaster that occurred off the coast of Brittany. Cleanup cost $100 million, but the worst effects are yet to be felt.

January 7 (PBS) National Geographic Society — **"Gold!"** looks at the precious metal from all angles — from how it's mined to how it affects our daily lives.

January 11 (PBS) NOVA — **"The Long Walk of Fred Young"** examines the conflicting worlds of a Navajo Indian who is also a nuclear physicist. Now working on the laser fusion project at the Los Alamos Scientific Laboratory, Young "has bridged the entire span of human technological development."

January 18 (PBS) NOVA — **"A World of Difference"** profiles the life of controversial behavior psychologist B. F. Skinner. More than 30 years ago Skinner introduced the climate-controlled crib in order to test his theory that environment controls behavior. His book *Walden Two*, published in 1948, grew out of his belief that humans could design a better society shaped by positive personal reinforcement. This program travels with Skinner to visit Twin Oaks, a rural, 11-year-old cooperative based on the book's ideals.

January 25 (PBS) NOVA — **"The Mind Machines"** is a repeat of last year's program looking at Artificial Intelligence. Although research in Artificial Intelligence was begun less than 30 years ago, the results are impressive. There are critics, however, and some claim that computers are not really capable of subtle human-like thought. Others fear that someday computers will outpace their creators.

January 28 (PBS) National Geographic Society — **"Hong Kong: A Family Portrait"** takes viewers to one of the most fascinating cities of the twentieth century and reveals it through the eyes of a single family.

January 28, 29, 30 (PBS) — **"The Energy War"** details the battle in Congress over the natural gas pricing bill.