To Choose Non-Toxic Technologies

New fossil fuel technologies will produce new pollutants, but viruses may provide quick screening tests for determining the health hazards of those chemicals

BY JULIE ANN MILLER

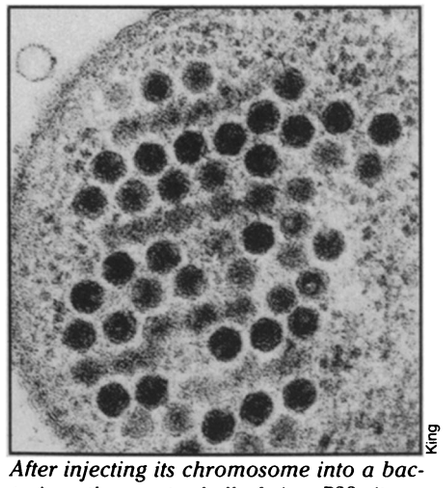
No matter what roles solar and nuclear energies play in the more distant future, there is little doubt that combustible fuels will power the immediate future. And already the push is on to develop technologies that will squeeze more energy out of oil and coal and to use forms of fossil fuels that until recently were too difficult or too expensive to produce. These new conversion techniques can help solve our energy problems, but in doing so they might contribute to human health problems by releasing a great variety of chemical byproducts into the air we breathe.

Because many of the chemicals expected as byproducts are already known to affect biological processes, several interdisciplinary projects aimed at developing new fuel technologies now include biologists and toxicologists, along with combustion engineers and analytical chemists. While the biologists may feel a little like spoilsports in the energy vanguard, their findings could help prevent selection of technologies that will in the long run prove harmful to human health.

"It would be tragic to have to wait thirty years to determine the standard mortality ratios of workers in the coal liquefaction industry in order to decide which effluents were most in need of control," Jonathan King told a recent conference in Park City, Utah, on Implications of New Energy Technologies.

King, a biologist at the Massachusetts Institute of Technology, is well known for his work on the structural details of viruses that infect bacteria. He is also known for his emphasis on the social content of laboratory research and for insisting that scientific research be used to benefit, not endanger or mislead, the public. This and his scientific expertise have led King to work in a new area called "molecular toxicology." He is trying to devise fast and inexpensive tests to indicate which chemicals released by industrial processes may endanger human health.

The prototype fast laboratory test is the Ames test for chemicals likely to cause cancer. Bruce Ames, another molecular biologist who wanted his research to have practical, beneficial applications, devised a method of detecting chemicals that make changes in the genetic material of bacteria. The test is simple, inexpensive



King

After injecting its chromosome into a bacterium, the empty shell of virus P22 sits on the surface (upper left). Within the bacterium, new viruses are filled with DNA.

and takes only a few days, whereas testing chemicals in animals can take years. Although bacteria are far removed from humans physiologically, the structure of DNA, the genetic material, is universal. Thus chemicals that affect bacterial DNA are good candidates for substances likely to affect human DNA and must be considered as possible carcinogens. Other simple tests for potentially cancer-causing chemicals are being developed rapidly. A variety of simple organisms and even human cells grown in laboratory culture are being put to use as early warnings of potential human hazard.

"Though cancer is a major cause of human disease and suffering, it is only one component of environmental and occupational disease," King explained in an interview in his Cambridge laboratory. He points out that the medical literature contains a wide variety of examples in which a chemical agent initiates disease: carbon tetrachloride in liver problems, mercury in kidney damage and cadmium in bronchial problems. Traditionally, however, physicians have shown far more concern with infectious agents.

Because King is an expert on how the individual parts of a virus assemble into complex biological units, he focuses on the assembly of parts in human tissue. In a healthy person, tissue is continually being replaced. If a toxic chemical were to poison that continual renovation operation, its effect would show up as a chronic, "breakdown" disease. King believes that much chronic heart, lung, liver and kidney disease may result from chemical compounds that disrupt cell replacement.

Biologists have only recently become aware of the complicated processes involved in the assembly of such cellular subunits as the membranes, organelles and cytoskeleton. "Cells are replaced in an extraordinary set of processes," King explains. But animal cells have no monopoly on extraordinary assembly. Even the simplest biological examples, the assembly of viruses that infect bacteria, are very complex. Years of research by King and others have produced detailed descriptions of how the many pieces fit together in those microscopic living puzzles.

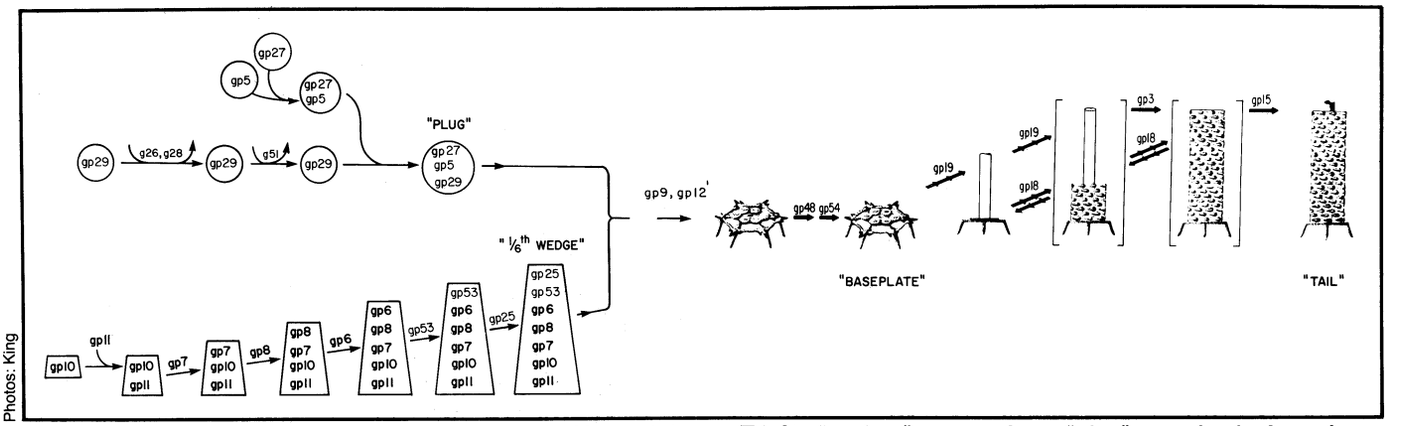
As bacteria provide an early alarm system for chemicals likely to cause cancer, so viruses may provide warning of chronic disease. King says, "Eventually we hope to be able to look at the chemical structure of a particular compound and be knowledgeable enough about its molecular toxicology to say 'Be careful, this compound is known to interfere with membrane assembly processes and will almost certainly be a problem.'"

The realization of that hope requires a more precise understanding of how environmental and industrial chemicals lead to disease. To pinpoint chemicals' interaction with the materials of life, King and colleagues are looking at the self-assembly of two viruses. Already they can isolate all the viral parts before they are assembled, mix them together in a test tube in the correct sequence and produce live, infectious viruses. If a chemical interferes with the assembly, its exact action can be quickly detected.

One virus under study is called T4, which infects *Escherichia coli,* the common intestinal bacterium. King and collaborators are using T4 to screen compounds that might inhibit the interaction of proteins in assembly processes. The virus T4 has a surprisingly complicated tail structure, which it uses to anchor itself to a bacterial cell and to pass its genetic material into the bacterium. Assembly of the T4 tail requires a sequence of at least 15 steps eventually combining 21 distinct proteins. The tail then joins a virus head, which already contains the chromosome. Because a gene on the viral chromosome has been identified for each tail protein, the T4 tail is considered the classical model for genetic control of biological assembly.

While T4 is the champion of tail assembly, a virus called P22 is useful for studying the process that packs the chromosome into the viral head. The interactions of proteins and DNA are crucial in the operation of all cells, bacterial or human, so knowledge gained from viruses in bacteria may be widely applicable.

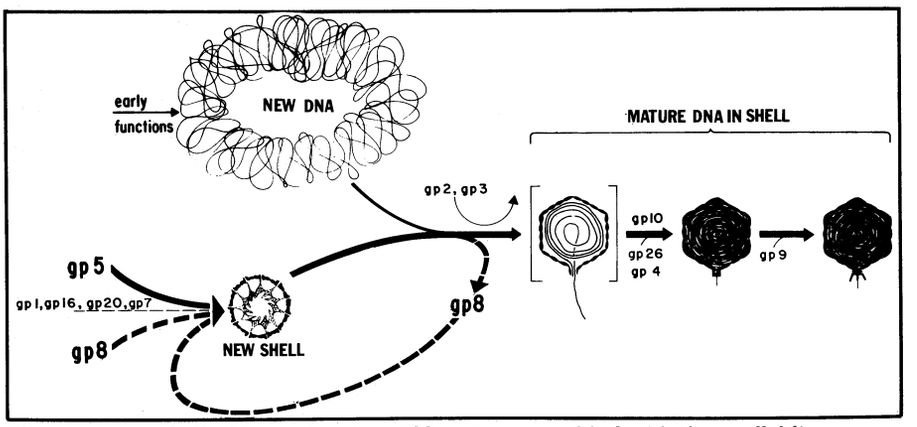
"It is very likely that the ways viral protein binds to viral DNA are similar to that mechanism of any protein," King says. "Chemicals that interfere with that binding site on proteins of a virus are good candidates for all such proteins."



Photos: King

A set sequence of steps assembles the complex tail of bacterial virus T4. Six "wedges" surround one "plug" to make the baseplate. Each participating protein is named for the gene that specifies it ("gp29" means the product of gene 29).

The virus P22 infects *Salmonella typhimurium,* the same bacterial species used in the Ames test. When the virus injects its chromosome into a bacterial cell, the viral chromosome directs production of new viruses. First a shell of the major coat protein forms, assembled with a scaffolding of a second protein. Next, with the participation of three other proteins, the scaffolding is ejected and a newly synthesized viral chromosome enters the coat. Then other proteins cap the shell and form part of the apparatus to be used later for injecting the chromosome into another bacterium. Finally, the tail spike protein helps the virus to attach to a bacterium. As in the T4 experiments, a gene for each protein in the assembly process has been located on the chromosome.



The precursor shell of bacterial virus P22 is first assembled with the scaffolding protein (gp8). Then the shell is filled with DNA *and additional proteins added.*

King and colleagues are now using P22 assembly to test a variety of chemicals associated with coal use, such as sulfur and nitrogen containing polyheterocyclic aromatic compounds. They then are identifying the step involved for each inhibitory compound. Harlee Strauss and Jerry L. Bryant Jr. have demonstrated that some heterocyclic compounds inhibit DNA packaging in P22, whereas others do not. Edward Loechler is examining exactly how the compounds bind to the viruses. The inhibitory compounds include some acridines, chemicals first extracted from coal tar and used as dyes and medicines.

A mature virus, as well as the maturing particle, can be a sensor for potentially harmful chemicals. King and Bryant have identified the means by which a heterocyclic compound, in the presence of light, inhibits mature P22 viruses from reproducing. King believes that these results suggest a cause for the skin and eye irritations that roofers and road workers suffer when they are exposed in sunlight to volatile chemicals from tar and pitch. A similar photosensitization can occur with certain drugs and cosmetics. King suggests that photosensitization might be an early component of some human skin cancer.

A chemical suspect in human photosensitization is an acridine derivative, the same chemical that inhibits DNA packaging in immature viruses. Acridine derivatives also inactivate mature viruses in the presence of sunlight. Because acridine derivatives bind between the bases of DNA, most investigators assumed that the damage caused by the chemical prevents the chromosome from directing new virus formation. Bryant and King find, however, that virus particles treated with acridine derivatives and sunlight are unable to inject their chromosomes into host bacteria, let alone produce more viruses. The researchers discovered that the two proteins required for DNA injection are grossly altered in those viruses. Furthermore, to be inactivated by an acridine derivative and light, those proteins had to be in a virus containing DNA. Thus King and Bryant suggest that the acridine derivative first binds to the DNA, then sunlight converts the acridine into the active state in which it damages the nearby proteins.

In an unusual step for biochemists, King and colleagues plan to test their viral findings in an experimental animal. "We recognize this is new terrain," King says. The scientists have already detected alterations when proteins from rat liver cell chromosomes were exposed to acridine derivatives and light. Next they plan to look at animal cells growing in laboratory culture and finally at living animals.

The long-range goal of this research is to increase the scientific bases for toxicology. Whereas some aspects of biochemistry have been well identified, others are poorly understood. The chemicals from coal combustion are a good set of compounds to use to develop new testing techniques, King says. His work has been sponsored both by the National Institute of General Medical Services and by the National Institute of Environmental Health Sciences, through the MIT program on Health Effects of Fossil Fuels.

"I don't think compounds from coal are very toxic. Human beings evolved exposed to these compounds in burning wood," King says. "We don't want to work with very poisonous compounds. We're not heroes." King expects such synthetic chemicals as halogenated hydrocarbons to be far more hazardous. Such compounds will be tested only after the test systems are operating reliably.

As new fuel and other industrial processes introduce into the environment hundreds of complex molecules — some more toxic, some less toxic, some harmless — it is not feasible to test them all in extensive animal experiments, but molecular toxicology may eventually identify those compounds likely to harm health. "We need better ideas of how things are likely to be problems," King says. "We hope the time will come when such molecular assays can prevent environmental and occupational diseases, which now we have to detect by a body count." □