# The New P4 Laboratories: Containing Recombinant DNA

In November, the National Institutes of Health (NIH) plans to open the first P4 laboratories certified for recombinant DNA research in this country. One of them, the mobile containment facility, is located on the NIH campus in Bethesda, Maryland; the other is a renovated biological warfare laboratory at Fort Detrick, Maryland.

Few scientists have had the experience of working in facilities like these, which are specially designed to prevent the escape of potential pathogens into the environment. Little research in the past, even with agents as infectious as the anthrax bacillus, needed such stringent physical containment measures as those now required for certain kinds of experiments with recombinant DNA. In fact, since the cessation in 1971 of biological warfare research, there has been so little need for high-containment laboratories that building them became something of lost art.

Learning the technology all over again has delayed the opening of the NIH facilities once scheduled for May of this year, but institute officials now say that the work on the laboratories is almost complete. The opening of the one at Fort Detrick, however, could still be postponed because of a lawsuit filed by a resident of Frederick, Maryland, where the fort is located.

Until recently, most molecular biologists could do just about any experiment in just about any laboratory. That situation changed dramatically in 1974 when scientists working with recombinant DNA initiated a self-imposed moratorium on some kinds of gene-splicing research. There were—and still are—fears that the experiments, which involve the tying together of genes from different species into recombinant molecules, might produce a new agent that would cause a serious epidemic in humans or somehow harm the environment.

Much of the concern centers around the use of *Escherichia coli*, a species of bacteria that normally lives in the human gut, as a host in which the recombinant molecules multiply. Investigators point out, however, that the laboratory strain (strain K-12) commonly used for recombinant DNA research differs considerably from the wild strains and is thought to be incapable of living in the human intestine. In fact, it is a safe bet that up to now far more effort has been expended in protecting the experimental bacteria from the environment than the environment from the bacteria.

Nevertheless, uncertainty arose when it became possible to introduce genes from totally unrelated species into *E. coli*, or into other bacteria or viruses, for that matter. No one could say for sure that this would convert a harmless organism into a dangerous one. But then no one could say absolutely that it would not.

An often acrimonious controversy consequently erupted over recombinant DNA research. Eventually, in 1976, the NIH adopted a set of "guidelines" for regulating the research. The guidelines, which may soon be enacted into law by Congress, rank different kinds of experiments according to the degree of potential hazard thought to be associated with them and specify the conditions under which they can be done. Increasing degrees of hazard require increasing levels of biological and physical containment. (Biological containment means the use of mutant strains of bacteria or viruses that cannot survive outside the laboratory.)

Adoption of the guidelines ended the moratorium, but some experiments still cannot be performed because the required degree of biological or physical containment cannot be achieved. For example, experiments done under P4 conditions, which is the highest of four levels of physical containment, require a specially engineered laboratory that is not supposed to permit the escape of microorganisms to the outside—not out the window, nor down the drain, nor through the exhaust system, nor in or on the laboratory workers. The NIH facilities will be the first such laboratories to open in this country. Meanwhile, investigators have to hold up any experiments requiring P4 containment.

Emmett Barkley, the director of the Office of Research Safety of the National Cancer Institute (NCI), describes a P4 facility as a containment system within a containment system. The primary barrier that separates the laboratory personnel from the bacteria or other agent under study is the glove box (class III safety cabinet in the official terminology); the secondary barrier is the laboratory itself.

Glove boxes are enclosed cabinets with neoprene gloves attached to ports in the glass fronts. All manipulations of the presumably hazardous material must be carried out within the cabinets, which are fitted with the necessary equipment, such as centrifuges, microscopes, incubators, and so forth. The glove box in the renovated Fort Detrick laboratory is actually a U-shaped series of cabinets; at the base of and along one arm of the U there are two levels, one above the other, of cabinets (Fig. 1).

To be certified for recombinant DNA research, a glove box must be virtually airtight. To test for airtightness, the cabinets are filled with an indicator gas until the interior pressure exceeds that of the exterior by an amount equivalent to the pressure exerted by 3 inches of water. At this pressure differential, the cabinets must leak less than 0.01 ounce of the gas per year. Barkley says that testing, sealing the suspected leaks, and testing again is a tedious business that takes 3 to 4 days because of the time required for the sealant to set.

When in use, the pressure inside the cabinet is kept at a level below that of the pressure of the laboratory, as a further protection against the escape of an agent from the glove box. The air pressure in a P4 laboratory is also negative with regard to the ambient pressure. In addition, the exhaust air from both the glove box and the laboratory must either be filtered through a high-efficiency particulate air filter capable of retaining 99.97 percent of the particles in the air, or it must be incinerated, or both.

Researchers gain access to the inside of the glove box by inserting their hands and arms into the gloves. Materials and equipment can be brought in and out of the glove box in any of three ways: through a double-door autoclave; through an air lock equipped with a vapor disinfectant device for items that cannot be heated; and through a dunk tank containing a solution of sodium hypochlorite for things that cannot withstand either of the other two treatments. The doors of the autoclave and air lock are interlocked so that both cannot be opened at the same time. Once the inner door of either has been opened, the sterilization cycle must be completed before the outer door can be released.

As long as the integrity of the glove box is maintained, the secondary barrier should be unnecessary, according to Barkley. The late Arthur Wedum, who was in charge of biosafety at Fort Detrick when it was a center for research on biological warfare, has even written, "As far as biohazard *outside* the laboratory is concerned, most secondary barriers are more for reasons of public relations than for anything else."

But the glove box is not foolproof. Barkley points out that the gloves are the weakest part of the system; they can tear or be punctured while the researcher is trying to inject an animal, for example. They are also clumsy to use, especially for delicate manipulations. For this reason, the NIH facilities will be staffed by technicians trained in the use of glove boxes. The laboratories will eventually be available on a limited basis to visiting scientists, but the visitors will not do the experimental work themselves; rather they will develop procedures to be carried out by the regular staff.

The autoclave may be the next weakest part of the system. Autoclaves have been known to malfunction, fail to sterilize, and cause contamination of the personnel or laboratory. However, there are indicator devices that will tell the personnel that something is wrong with the autoclave.

Because of the possibility that an agent might escape from the glove box, the laboratory is equipped so that all exhaust air is filtered through high-efficiency filters and all liquid and solid wastes are sterilized. To prevent personnel from carrying a hazardous agent out of the laboratory, they must enter through a changing room where they replace their street clothes with protective clothing. And when they leave they must shower before changing back to street clothing.

John Richardson, biosafety officer of the Center for Disease Control (CDC) in Atlanta, describes P4 containment as a giant step above P3, the next lower level. Although P3 laboratories have some features in common with the P4 type, including restricted access, negative air pressures, and safety cabinets with filtered exhaust air, the former do not have provisions for sterilizing the laboratory exhaust or liquid wastes from laboratory drains. However, all materials used in experiments, whether performed in P3 or P4 laboratories, have to be sterilized before removal from the laboratory.

There are several P3 facilities at research institutions around the country, but few facilities comparable to the new P4 laboratories exist for the simple reason that very little research requires that high a level of physical containment. Richardson says that fewer than a half-dozen groups of investigators are doing work requiring maximum containment, and they are working with such organisms as the lassa and Marburg viruses that cause lethal diseases for which there are no treatments.

Even during the days of biological warfare research at Fort Detrick, relatively few kinds of procedures needed the highest level of containment. Everett Hanel, who has been a biosafety officer there for many years, says that those that did all involved large quantities of a pathogen in a highly infectious form. Examples include experiments in which animals were exposed to aerosols of the agent, or operations in which large batches of the material were produced, especially when it was dried to form a concentrated powder. But more ordinary research to study the biochemistry or genetics of a pathogen relied on lesser levels of containment plus good microbiological technique to prevent infections.

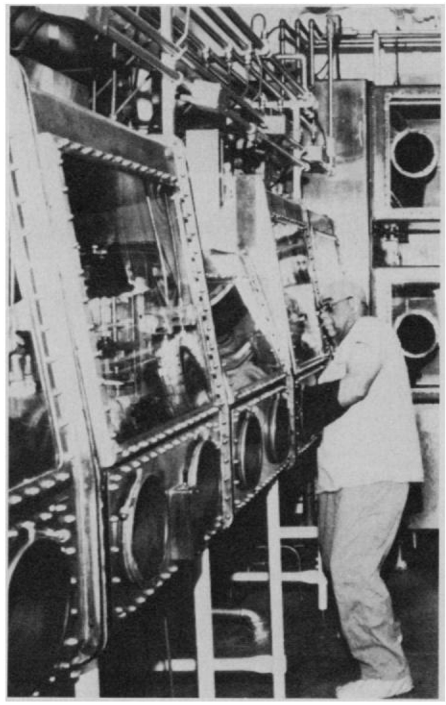

Fig. 1. The glove box used in the renovated P4 laboratory at Fort Detrick.

The NCI took over the germ warfare laboratories at Fort Detrick in 1971 for the study of cancer viruses. Although several viruses that cause cancer in animals or are suspected of doing so in humans need to be studied in P3 conditions with safety cabinets, none require P4 containment. This high level is specified only for an as yet hypothetical virus proved to cause cancer in humans.

Since the biological warfare work at Fort Detrick stopped in 1971, the building and maintenance of high containment facilities has become a lost art, according to John Nutter of the National Institute of Allergy and Infectious Diseases (NIAID), who is the project manager in charge of setting up the P4 facilities. The technicians, shopworkers, and other craftsmen who worked at Fort Detrick have dispersed, and renovating the laboratory there was a learning experience for all concerned. This situation no doubt contributed to the delays in finishing the laboratory which was originally scheduled to open in May of this year.

However, the certification procedures are almost complete and NIH officials expect to have the laboratories ready for occupancy sometime in November. The mobile containment facility on the NIH campus will be used only by NIH personnel, but the Detrick laboratory will be available for limited use by researchers from outside the institutes roughly 6 months after it opens. Experiments to assess the risks of certain types of gene-splicing manipulations that are to be carried out by Wallace Rowe and Malcolm Martin of NIAID have priority; other experiments will not be permitted until these are well under way.

Nutter concedes that there is no doubt that the demand for time at the laboratory will be greater than the supply. Officials at NIH are now working on the procedures for allocating the available time equitably. The situation should eventually be alleviated, although not solved, by the renovation of another high-containment building at Fort Detrick for use as a national center for doing recombinant DNA research requiring P4 containment. This renovation is still in the planning stage, however, and it will be at least 2 years before it is ready. All in all, molecular biologists are going to gain a better appreciation of the frustrations long suffered by astronomers and physicists who have to wait in line for the telescope or high-energy accelerator.

Moreover, a lawsuit filed by Ferdinand J. Mack, Sr., a lawyer and Frederick resident, could hold up the start of the risk assessment experiments. Mack filed the suit to halt the renovation of the laboratory on the grounds that NIH had failed to file an environmental impact statement (EIS) for the research to be conducted there. The National Environmental Policy Act of 1970 requires that an assessment be made of any federal activity, including research, that might adversely affect the environment.

In an out-of-court settlement, Mack and the NIH agreed that the renovation could proceed provided that NIH file an EIS covering recombinant DNA research performed in accordance with the guidelines. That statement is now awaiting approval from the office of Health, Education, and Welfare Secretary Joseph Califano. The agreement also stipulates that NIH give Mack 30 days notice before experiments begin in the Frederick facility.

Rudolf Wanner, who is responsible for preparing NIH's environmental documents, is doing an additional analysis of the risk assessment experiments themselves even though they are in conformity with the guidelines and a separate analysis would not normally be required. Wanner says that the benefit of doing the environmental assessments is that it puts on Mack the burden of proof to show that the analyses are incomplete or inaccurate.

Mack sounds as if he is prepared to do just that. The lawyer says that all he wants from NIH is an objective and fair EIS, but he doubts that he will get one because the NIH staffers preparing the statement are supporters of the research.

Despite his attempt to halt renovation of the laboratory, Mack is not worried that the physical facilities themselves are inadequate to do the containment job for which they were designed. Rather, his main concern is that human error will result in the release of some pathogen into the community where he lives. He contends that it is all right for scientists to decide to take a risk for themselves but it is not fair for them to impose the same risks on the community at large.

In contrast, both NIH and CDC officials maintain that in the long history of microbiological research, much of it with pathogens, there has been little if any documented evidence for the spread of infections acquired in the laboratory to the community. However, in the past infections have been acquired by laboratory personnel and, on occasion, by visitors. Thus, the fervor of the debate over recombinant DNA research makes it likely that a sharp watch will be kept over the operation of the new P4 facilities.—JEAN L. MARX

# Carbon Dioxide and Climate: Carbon Budget Still Unbalanced

The continuing growth in consumption of fossil fuel has raised the possibility of global climatic changes induced by carbon dioxide. Accurate predictions of such changes are hampered partly because a basic understanding of the production, consumption, and storage of carbon on the earth still eludes researchers. Most investigators in the diverse fields involved agree that more $CO_2$ is being produced than can be reliably accounted for, but there is broad disagreement as to how much is missing. Researchers also cannot agree about where it is most likely to be found. The controversy is one aspect of the debate on the long-term reliance on coal versus energy sources producing no new $CO_2$ such as nuclear, solar, and biomass sources.

The amount of $CO_2$ in the atmosphere has been observed to increase steadily since a monitoring program was begun in 1958, but the increase is equivalent to only about 50 percent of the $CO_2$ known to have been produced by fossil fuel burning in the same period. Until recently, it had been widely accepted that part of the remainder entered the oceans and part was taken up by terrestrial green plants. Now, however, some biologists are maintaining that it is not likely that any of the excess $CO_2$ is stored in land plants, and some researchers even contend that the land is a significant source of $CO_2$. The obvious conclusion that all the excess $CO_2$ is in the oceans is, however, disputed by many oceanographers. They maintain that the ocean cannot take up $CO_2$ fast enough.

The importance of the debate is that the future rate of increase in atmospheric $CO_2$ depends upon the relative importance of the various processes supplying it as well as those removing it. While the rate of increase over the last 20 years has paralleled the increase in fossil fuel consumption, the suggestion of deforestation as a major source of $CO_2$ has complicated the prediction of future levels.

George Woodwell of the Marine Biological Laboratory at Woods Hole is a leading American proponent of the idea that deforestation of the land creates a $CO_2$ source. He has attempted to estimate the amount released during the destruction of forests over and above that taken up by the regrowth of vegetation. His calculations, as do all others, depend on limited data gathered from relatively small geographical areas for extrapolation to a global scale. These data include observed rates of clearing of tropical forests in one region of Venezuela and in two regions of Brazil. The use of assumptions about the fraction of cleared vegetation that is converted to $CO_2$ and the rate of regrowth of the forest allows an estimation of the net amount of $CO_2$ released from tropical forests each year. The tropical forests represent the largest mass of carbon in land plants. Temperate and northern forests have also experienced regrowth after extensive exploitation. Allowance for this uptake was based on reforestation data for the state of Maine. Yet another reservoir of carbon is to be found in soil organic matter which contains several times the carbon in living plants. Woodwell believes, on the basis of field data primarily from South America, that losses to the atmosphere from this source have been enhanced because of increased exposure by cultivation and deforestation.

He and a number of his colleagues estimate that an amount of carbon equivalent to probably 80 to 160 percent of the fossil fuel $CO_2$ is being released as the net result of deforestation. Although such calculations represent "an educated guess," Woodwell contends that "we know a great deal about forests and a good deal about succession," the progressive regrowth of cleared land.

Other investigators have developed similar if less extreme estimates following the same general reasoning. Bert Bolin of the University of Stockholm estimates that releases from the living and dead organic matter (the biota) on land equal 10 to 35 percent of the fossil fuel contribution. C. S. Wong of the Institute of Ocean Sciences, Victoria, British Columbia, sets an upper limit of about 30 percent for the biotic contribution. Woodwell believes that no lines of reasoning currently under consideration suggest that the biota is taking up more $CO_2$ than it releases, or in other words, that it is acting as a sink.

These tentative findings appear to exacerbate the problem of the missing $CO_2$. Rather than providing a storage place for approximately 20 percent of the fossil fuel $CO_2$, the land biota would be a significant source of $CO_2$. In the worst case, a storage place for three times the quantity of carbon previously considered would need to be found. Although proponents of such a source do not know where the excess $CO_2$ goes, they often suggest that it probably ends up in the ocean.

Oceanographers dispute the assertion that such large quantities of $CO_2$ could be absorbed by the ocean. They point out that the ocean as a whole is a relatively sluggish body of water. Its surface waters, which are only about 75 meters thick, are well stirred and free to exchange $CO_2$ with the atmosphere. But these shallow waters cannot store the necessary quantities of $CO_2$. The deep waters, which constitute the bulk of the oceans, circulate very slowly. This is important because the use of fossil fuels has been so concentrated in recent years that the average age of all the fossil $CO_2$ released to date is 28 years, a period in which only a few percent of the deep water exchanges with surface water.

The warmer suface layer is separated from the cold, deep water by a region known as the thermocline in which temperature decreases rapidly with depth. The thermocline acts as a barrier be-