Recombinant DNA meets the Cambridge City Council

In a move seen more as a bad precedent than a real impediment to research, the Cambridge, Mass., city council imposed a moratorium on moderate and high risk recombinant DNA experiments at Harvard and the Massachusetts Institute of Technology last week. No current research will be stopped or postponed by the three month moratorium, but researchers there and elsewhere are worried by the broad implications of this action.

It is not surprising that the first instance of community control over the new gene transplant technology was precipitated in Cambridge. That city is both the seat of intensive recombinant DNA research and of intensive opposition by radical scientists, based on the potential hazards of the work. There has been, too, a history of sniping by Cambridge Mayor Alfred Vellucci—a favorite among blue-collar workers—at the two wealthy universities.

The city council also established a review board of scientists and citizens to recommend, after a three-month study, a policy for the city to follow. The city could declare the moderate and high-risk experiments health hazards if the review board so recommended. Measures needed for safe containment of these so-called P_3 and P_4 experiments (as well as the less hazardous P_1 and P_2 experiments) have been established by the National Institutes of Health in extensive guidelines published June 23 (SN: 7/3/76, p. 3).

An attempt by Harvard to build a moderate-risk (P_3) facility led to the recent action. A Harvard recombinant DNA researcher requested funds from the National Cancer Institute to assist in the renovation to the P_3 level of the fourth floor of Harvard's biology laboratory building. A small part of the space would be used for cloning and gene transplantation work. The rest would accommodate traditional cancer research work such as tissue culturing and virus experiments. The university approved the project, and NCI agreed to fund 75 percent of the $600,000 cost.

A dispute broke out among biology faculty members over the safety of renovating an old, "vermin-infested" building for potentially hazardous experiments, and was sparked to a major battle by Science for the People members and other opponents of the new technology. Mayor Vellucci read about the dispute, called a city council meeting to discuss the matter, heard testimony from both sides, then introduced a resolution to prohibit all recombinant DNA experiments in Cambridge for two years. The council at their July 8 meeting, compromised on a three-month moratorium on only the higher risk experiments and the establishment of a review board.

The moratorium will not affect ongoing research at Harvard. There is no P_3 facility, and under NIH guidelines, moderate risk experiments cannot be conducted without one. The council's ruling did not refer to the building plans, and both NCI and Harvard are moving ahead with the renovation.

Research at MIT probably will not be affected either, at least during the next three months. That university does have a P_3 laboratory, and several biology researchers, including Phillip Sharp, have plans to conduct moderate risk experiments. But MIT's biohazard committee had been waiting for the final NIH guidelines and hasn't yet certified the facility as P_3 according to the new definitions. "The three-month moratorium time," Sharp says, "will probably be taken up as we wade through the bureaucratic process of getting the facility certified. If there had been more of an impact, the universities would have reacted more strongly."

Many individual researchers, however, have reacted strongly to what they see as a very bad precedent for recombinant DNA research and other more traditional areas of inquiry as well. Paul Berg of Stanford University sent a strongly worded letter to Mayor Vellucci and the city council when the resolution for a two-year ban was introduced. Berg is a pioneer in the new field and was one of the researchers to call for a moratorium on some forms of the work among scientists in 1974, until a conference could be held at Asilomar, Calif. in February 1975 (SN: 3/8/75, p. 148).

In his letter, Berg stated: "Many scientists and laymen alike are deeply concerned that the Cambridge city council is considering suppression of a serious and responsible search for new knowledge. The implications of such action are ominous indeed. What additional forms of legitimate and worthy inquiry—scientific, artistic, political—will self-appointed vigilante groups next condemn on the pretense of imagined risk?"

Committees of the NIH spent 18 months preparing guidelines sufficient, Berg says, to contain the potential risks inherent in the work and more stringent, in some cases, than evidence dictates is needed. "The city council," he said in a telephone interview, "lacks that kind of expertise, and has stepped in on the basis of a few critics who dredge up risks—some imaginary and all extremely unlikely—on very little evidence." The mayor and the strongest opponents, he says, seem to have motives other than safety and also seem to find that issue an expedient route to public attention.

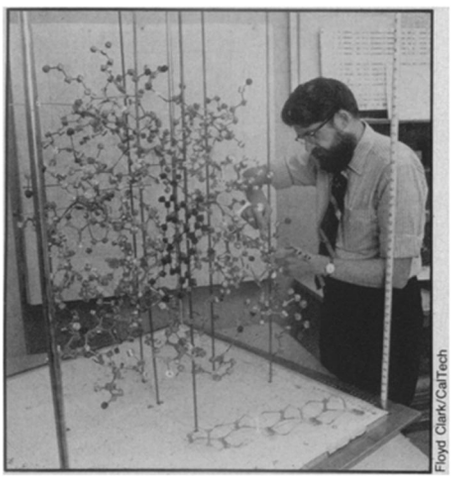
One NIH scientist called the ruling "ludicrous," since researchers at Harvard Medical School, Brandeis and Boston Universities (all located in Boston) can still do P_3 and P_4 experiments with the proper facilities. "If there were a real biohazard," he said, "it clearly wouldn't respect municipal boundaries. How can they possibly feel secure if they are really worried that a lethal bug could be produced?"

A similar attempt to build two P_3 laboratories at the University of Michigan began in January 1975, and ended two months ago, after long and heated debate within the university community. Several committees of university scientists and nonscientists approved the proposed facilities but a small group of faculty members who oppose the research asked that the Board of Regents hold up funds. A series of debates, meetings and public forums followed, during which the Ann Arbor city council was urged to become involved. That council agreed, however, only to ask NIH for an environmental impact statement, then eventually tabled the motion. The Regents approved the building in late May. □

Enzyme clue to earliest evolution

By carefully noticing how certain characteristics, such as color, varied among different plant and animal species, Charles Darwin was able to develop a picture of how the species had evolved. More recently, variations of protein structure have been used to refine the theory and push back knowledge of the origin of the species to very simple unicellular organisms. Now, a 12-year study of one such protein, the enzyme "cytochrome c," has revealed important clues to the evolution of the very earliest life forms, some 3.5 billion years ago.

Cytochromes are responsible for transferring electrons in plant or animal metabolism, enabling the organism to convert energy from one form to another. Since they are fundamental to the functioning of some of the earliest microorganisms, CalTech physical chemist Richard E. Dickerson and his associates began an extensive study of the complex structure of the 2,000-atom molecule to determine variations among different primitive species. The research has resulted in a



Dickerson builds cytochrome c model.

seven-step scheme of earliest evolution.

Though remote, this early evolutionary process was critical for the development of the rest of life, for it started with an atmosphere that contained little or no oxygen, 3.5 billion years ago, and ended with the development of multicelled organisms in an oxygen-rich atmosphere 1.2 billion years ago. Thus, the evolving bacteria and algae not only laid the genetic foundation for the higher forms of life to come, but were responsible for changing the atmosphere to make their development possible.

The research into such an early process is possible only because barely changed descendants of even the most primitive organisms still exist. The first bacteria, whose descendants are typified by the organism that causes gangrene, were able to obtain energy only through the inefficient method of fermentation. The first big improvement on this process occurred about 3.0 billion years ago with the evolution of "sulfur" bacteria, which contained the first cytochrome enzymes and could conduct photosynthesis using the hydrogen sulfide then present in the atmosphere. These were followed by bacteria that could utilize more energetic sulfate molecules. (Their descendants are found today in sewage.)

The next big step was development of the Krebs cycle, the respiration process that produces the universal "energy currency" of life—the ATP molecule. The beginning of green plants, as we know them, then came with the development of microorganisms with the ability to use water instead of a sulfur compound in photosynthesis. These were the ancestors of blue-green algae, and over the next hundreds of millions of years they slowly produced our present oxygen-laden atmosphere. Cytochrome c was fundamental to both the new respiration and photosynthesis processes.

But then comes a missing link. A unique class of bacteria is thought to have evolved that were capable of both nonsulfur photosynthesis and oxygen respiration based on the Krebs cycle. Such an organism would have evolved from bacteria that had to derive energy from the sun, through photosynthesis, and would have led to the final great stage of bacterial evolution—the organism that could rely solely on oxygen respiration. These oxygen respirers, which evolved some 1.8 to 1.4 billion years ago, were able to extract 19 times more energy from the same amount of food as the primitive fermenting bacteria.

The pace of evolution was by then able to speed up considerably, to produce nucleated cell organisms some 1.4 to 1.2 billion years ago and eventually multicelled organisms about 1.2 billion years ago. At this time, the plant and animal kingdoms began to diverge. Later stages of evolution have been well understood.

An important conclusion Dickerson derives from this scheme is that respiration evolved from photosynthesis, with the electron-transporting enzymes being the part common to both systems. Cytochrome c, for instance, occurs near the end of the transport chain, delivering an electron to an oxidase molecule in respiration, and to a chlorophyll molecule during photosynthesis.

Though Dickerson's research has involved analysis of samples taken from present living organisms, fossil and geological records corroborate his findings. Fossil spheres resembling blue-green algae have been found in South African rocks 3.1 billion years old. This means that a relatively short period of less than 1.5 billion years may have elapsed between the condensation of the earth and the emergence of life at the bacterial level, Dickerson concludes.

Several reports have been published of this decade-long project, and one of the most recent and comprehensive appears in the JOURNAL OF MOLECULAR BIOLOGY, (vol. 110, p. 473). □

Heart attacks and testosterone

Despite the well known fact that men are more susceptible to heart attacks than women are, at least before women go through the menopause, scientists haven't been able to produce any hard data explaining why. Now some tough evidence is finally being served up by researchers at Georgetown University Medical Center in Washington. The culprit? The male steroid hormone testosterone.

A thrombus is a blood clot that forms inside a blood vessel and obstructs it, often leading to a heart attack or a stroke. An ingenious method was developed in 1973 to produce in rats experimental arterial thrombi that are similar to those that occur naturally in humans. A long plastic loop is inserted into the abdominal aorta, a major artery. With the passage of time, platelets in the blood start to bind together to form a thrombus and obstruct the blood vessel—a natural response to this foreign object in the cardiovascular system. The Georgetown investigators used this technique to study the effects of testosterone on thrombosis and thrombosis-caused death.

They found that young male rats have twice the thrombus size and thrombosis death rate of young female rats. However, the size and death rate increase comparably in older male and female rats. These results dovetail with the clinical situation where young men are more susceptible to heart attacks than young women are, yet where thrombosis increases with age for both males and females.

Assured that the rats' susceptibility to thrombosis was comparable to the human situation, they next tested the effects of testosterone and a female estrogen on thrombosis and thrombosis deaths in rats. One group of male and female rats received injections of testosterone, then thrombi were induced in them. Another group of male and female rats received injections of an estrogen, then thrombi were produced in them. Still a third group of male and female rats served as controls (received no injections prior to thrombus induction). The testosterone markedly increased the rates of thrombus formation and increased the death rate from thrombosis more than fourfold in both male and female rats. This result strongly indicts testosterone as a causative or conspiratorial agent in thrombosis and thrombosis-caused deaths. In contrast, the estrogen treatment did not exacerbate thrombosis or thrombosis death rates in either male or female rats. In fact, it even decreased thrombus weights in the majority of male rats.

Finally, the researchers studied the effects of an antitestosterone agent in both male and female rats submitted to experimental thrombosis. This agent, Flutamide, significantly decreased thrombosis death rates in both male and female rats when they received it along with testosterone. Specifically, of the rats treated with testosterone alone, 62.5 percent of the males and 34.3 percent of the females died. When the antitestosterone agent was given with testosterone, 33.3 percent of the males and 16.7 percent of the females died.

"Our results provide for the first time significant experimental evidence for an association between sex and age and the development of arterial thrombosis," the Georgetown team explains in the June 24 NATURE. Very clearly, the male hormone testosterone is a high risk factor, they conclude.

They hope that their findings will give impetus to the search for antitestosterone agents that would not feminize men yet still offer them protection against heart disease. □