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RECOMBINANT DNA

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PROMISE AND HAZARDS OF RECOMBINANT DNA RESEARCH: THREE SCHOOLS OF THOUGHT

Molecular biology has reached a stage that is brilliant with both danger and promise: the stage at which genetic material from one organism is being placed within another so that the inheritance of the host is changed to reflect the characteristics of the donor. Thus the genetic barriers between the species are being probed and, in a first few simple cases, surmounted so as to produce hybrid creatures.

Few doubt that this technology has the potential for deliberate misuse to produce great dangers. Genes from disease causing (pathogenic) organisms, or from organisms that produce highly toxic agents, could be implanted in hosts capable of rapid spread so as to produce dramatic new biological dangers. Not only common sense, but the biological treaty of 1972 to which the U.S. and 110 nations have become signatory, demands that scientists eschew development of such agents. Nevertheless, since treaties are neither universal nor self-enforcing, the world must begin to face a biological proliferation threat that might before long, rival that of nuclear weapons.

This new danger would, by itself, militate for a halt to this research were it not for the promise implicit in these techniques. Five percent of the population suffer from a few thousand different genetic diseases which may, in time, be amenable to future genetic engineering. The underdeveloped world lives in perpetual risk of famine; vast improvements in agriculture should be possible with genetic engineering of plants. A rising cancer rate already affects one person in four and these new techniques represent the single most important tool in many years for the investigation of the underlying, and hence highly relevant, biological processes. Ultimately, a more pre-

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SPLITTING ATOMS AND TRANSPLANTING GENES

With the first beginnings of practical genetic engineering, the biologists have begun to displace the physician in the spotlight of public concern. There is every indication now that society will have continuing and grave difficulties digesting biological advances.

With nuclear weapons, the danger was at least stark, and the solution clear: refrain from use of nuclear weapons. Deterrence now seems relatively simple. Genetic engineering, by contrast, begins with an unprecedented and worrisome problem: the accidental escape of organisms, unknown in nature and potentially, or even known to be, dangerous. It moves on to the problem of inadvertent by-products of biological successes as in the query: will the plants that are taught to fix their own nitrogen generate, somehow, a tenacious weed? Later, if we are sufficiently careful to avoid misusing this new power over the ecology, we may move on to solving cancer and the genetic diseases of man — and ultimately to the improvement of man. No doubt in certain cases, determining who will secure the benefits of these cures and, later, which, if any, improvements to strive for, will occupy the world's attention for generations.

Hovering over these problems of societal absorption is the clear danger of deliberate misuse, for biological weapons, of techniques that may rapidly proliferate not only to one hundred eleven nation states but even to individual researchers. In the nineteen forties many contemplated the danger of an unprecedented nuclear arms-race and concluded that the world would not survive the nuclear threat in a state of relative anarchy among nation states. One is tempted to reach the same conclusion again in the biological arena—even while the first nuclear conclusion is still being perilously tested.

The researchers have behaved with unprecedented restraint and caution. Raising the issue themselves; bringing it to public attention; urging the voluntary deferral of various experiments; and debating the hazards in full public view, represents four quite different and thoroughly commendable steps. In addition, most have, quite surprisingly, been able to come to agreement on a set of guidelines that have grown steadily more stringent — even while many of the researchers have grown more sanguine about the dangers. This is a tribute to the statesmanship of their leaders. It is no surprise that now they want to go ahead with research which all observers agree is filled with promise, and which promises tremendous assistance in understanding biology. They only ask a "yellow" light —the right to proceed with caution.

However, some researchers and observers whose judgment FAS respects, still have different views on these matters. And despite intense thought by the molecular biologists, the issue does seem to have been considered somewhat unimaginatively thus far. The possibility that relevant creative ideas may yet exist impells us to request members to write expressing themselves on this problem.

FAS, founded by physicists, has worked for thirty years to control the implications of the fact that the atom could be split; now diversified to include many biologists, FAS pledges no less steadfast monitoring of the public policy implications of a fact no less far-reaching — the feasibiilty of transplanting genes. □

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cise knowledge of cellular processes would also permit a new attack on many other cellular diseases, ultimately, genetic engineering may hold forth the risky potential of improving man himself.

In order to achieve this promise, there will be required long periods of intensive work in many laboratories, in areas of biology in which there is necessarily great ignorance. As a result, the possibility of inadvertent creation of biological dangers cannot be dismissed. As the attached newsletter reveals, an unprecedented, constructive and wholly admirable effort to devise safeguards has been going forward among the researchers themselves since 1973. Notwithstanding the construction of a broad and impressive consensus among them, there remain significantly different approaches to this problem among equally informed molecular biologists.

The first school of thought either considers the proposed guidelines to be overly restrictive or has acceded to them reluctantly with the thought that the public would otherwise demand even more. This view considers inadvertent dangers wholly speculative and exaggerated. It sometimes fears restraints on free inquiry. It sees anomalies in the way in which other societal dangers, considered to be comparable, are tolerated — even such closely related dangers as the work by fellow biologists on known-to-be infectious organisms.

The second school emphasizes the speculative quality of the danger and believes that a system of regulating degrees of hazard by degrees of physical and biological methods of containment is both appropriate and feasible. (Biological containment involves developing strains of well-known organisms so enfeebled that they will not survive outside the laboratory should they escape at all.) This approach supports the guidelines. Along with many representatives of all three schools, it fears its proposals will be unreasonably perverted into more rigid regulations: difficult to amend; increasingly seen to have contradictions as research progresses; and, finally, destroyed by internal contradictions and quiet scientific revolt.

There is a third school that sees real danger in the multitude of things that might go wrong. This school is particularly disturbed that the experiment would use the widely studied E. coli bacteria which can live in the gut. By focusing their attention so singlemindedly on this organism, biologists have made headway in understanding fairly completely at least one species. But this organism, in which so much research has been invested, is now seen as far too ubiquitous, and promiscuous in exchange of genetic material for dangerous experiments. Is it sufficient to enfeeble strains that can live in man? Or should several years be spent working up the same degree of knowledge in some organism occupying a much more obscure ecological niche? In the meantime, or in general, this view often recommends that much more of this research be done in the kind of maximally secure laboratories previously for biological warfare experiments; more of these would be built.

This school sees no issue of "freedom of inquiry" but rather "freedom of manufacture" of what might become public health hazards. It sees no great hurry in pushing ahead in areas where eventual successes will last forever. And it sees not only health hazards but hazards to sustained research if something should go wrong.

We have developed no consensus upon the two interrelated, critical questions of public policy: what degrees of precaution do public health and public confidence demand and how they can be achieved? Because the issue is so important, we believed that we must carry it to you in an effort to develop a consensus. We ask for reader comment by letter and through a ballot on page 9. □

— Reviewed and Approved by the FAS National Council

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SOME SAMPLE BENEFITS

Robert Sinsheimer reports:

"It is very probable that in time the appropriate genes can be introduced into bacteria to convert them into biochemical factories for producing complex substances of medical importance: for example, insulin (for which a shortage seems imminent), growth hormone, specific antibodies, and clotting factor VIII which is defective in hemophiliacs. Even if these specific genes cannot be isolated from the appropriate organisms, the chances of synthesizing them from scratch are now significant.

"Other more grandiose applications of microbial genetic engineering can be envisaged. The transfer of genes for nitrogen fixation into presently inept species might have very significant agricultural applications. Appropriate design might permit appreciable modifications of the normal bacterial flora of the human mouth with a significant impact upon the incidence of dental caries. Even major industrial processes might be carried out by appropriately planned microorganisms."

As examples of industrial processes, A. M. Chakrabarty of General Electric has experimented with microorganisms that have special affinity for binding precious metals, such as gold or platinum, with a view to using them to recover these substances from industrial wastes, seawater, or even from ores. With regard to a second application — cleaning up oil spills — he notes that existing bacteria have the capability to degrade hydrocarbons but usually only one kind of hydrocarbon each; hence only one component of the spill is attacked. When one uses several strains of bacteria, they interact so as to destroy each other. Genetic engineering could continue to improve the digestive range of a single bacterial strain. (At least one scientist has observed that the required release of such a bacterium might be dangerous to the reliability of gas tanks.)

Since the microorganisms are, by digesting the oil spills, converting hydrocarbons into protein, this process could also be used to convert petroleum into protein as a source of food. In theory, the food supply could also be attacked directly by enhancing the ability of animals (or even man) to digest cellulosic foodstuffs, such as grass and weeds.

It has been observed that one might locate and isolate the genes on disease-causing virus that produce the virus' hard protein coat. It is the coat that is the antigen which produces in turn the antibodies that protect the human against the disease. Hence these isolated genes might be used to produce bulk quantities of the protein coats which could then be used as vaccines.

Other suggestions include improving the efficiency of photosynthesis to improve crop production or enhancing the nutritive value of plant products. □

HAZARDS OF INADVERTENCE

The basic current hazard is the introduction into bacteria of genes which make the bacteria more dangerous. In the simplest case, such genetic changes might give one strain of bacteria the resistance to antibiotics that exists in other strains; thus some such antibiotic as penicillin might suddenly find that strains of bacteria that cause pneumonia had become resistant to its application.

A still more dangerous case would occur if E. coli strains were provided with a genetic means — possibly carried on the very plasmids being used to transfer alien genes into E. coli — to cause disease directly. In general, foreign fragments of DNA introduced in various ways into bacteria might inadvertently carry other genes in addition to those the researcher intended to introduce.

Experiments using virus that can cause tumors are especially dangerous since the tumors may take decades to arise; in theory, at least, bacterial strains carrying such tumors introduced from viruses might do their deadly work for many years before it was even recognized that an accident had occurred.

It is also feared by some that biological safeguards involving the enfeeblement of test organisms might not be adequate, in general, because the original cloning culture might be contaminated, or because the enfeebled host bacteria might die only after transferring its DNA to another organism; in effect recombinatory events might defeat the safeguards.

One observer notes that animal genes to be transferred into bacterial must be well-characterized lest latent cancer-causing genes be transferred inadvertently:

"One hypothesis of carcinogenesis envisages the integration of a latent form of oncogenic viral DNA on the animal cell DNA. Under normal conditions, the

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ASHBY REPORT CONCLUSIONS

Benefits

We reiterate our unanimous view that the potential benefits are likely to be great. The most substantial (though unpredictable) benefit to be expected from the techniques is that they may lead to a rapid advance in our detailed understanding of gene action. This in turn might add substantially to our understanding of immunology, resistance to antibiotics, cancer, and other medically important subjects. Furthermore, application of the techniques might enable agricultural scientists to extend the climatic range of crops and to equip plants to secure their nitrogen supply from the air. Another possible application is that segements of DNA, selected because they are templates for valuable products such as hormones, antigens or antibodies, might be produced in bulk by multiplying them in cultures of E. Coli: this would be of great benefit to medicine. And it is not inconceivable that the technique might ultimately lead to ways to cure some human diseases known to be due to genetic deficiency.

Hazards

Such new combinations might although this is only speculation extend the host-range o fa disease from animals to man, or provoke malignant cell growths, or confer new patterns of resistance to antibiotics. However, it would be an excessive constraint upon important work to insist on conducting all such experiments with the elaborate and expensive procedures used for the most dangerous pathogens. Somebody therefore has to match the scale of precaution to the estimates of risk.

– Report of the Working Party on the Experimental Manipulation of the Genetic Composition of Micro-Organisms, Chaired by Lord Ashby, presented to the British Parliament, January, 1975

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viral genes are not expressed. Treatment with certain forms of carcinogenic agents may trigger the latent genes, thereby releasing the harmful virus.

"There is a paucity of knowledge about the sequence of genes on the eukaryotic chromosomes so that introduction of any sequence of genes from higher animals, if not well characterized, may lead to accidental introduction of latent viral genomes inside the bacterial cells. If the regulatory genes that prevent the expression of the viral genomes are not co-transferred, there might be the real danger of such viral genomes becoming fully functional inside the bacterial cells. The bacterial cells may, therefore, virtually become carriers of such infectious agents."

Over and above these dangers of incorporating disease causing or drug resistant genes into bacteria, some believe that there are dangers associated with *any* incorporation of genes from higher organisms into bacteria. It is argued that the products of such incorporation will inevitably escape — laboratory procedure and the abilities of E. coli to recombine genetic material being what they are — and that the escaped organisms will inevitably become established in the world of microorganisms.

What could happen? It is important to understand that, while all living things use the same genetic code, there are very fundamental differences between the single-celled bacteria (prokaryotes) and the cells of higher organisms (called eukaryotes). Although the cells of lower and higher organisms are interacting intensely, they do not seem to exchange genetic material in nature; hence the introduction of eukaryote genes into prokaryotes is an activity in which, apparently, nature has not engaged.

Genes Can Be Transmitted Without Being Expressed

In this regard, it is highly significant that the higher organism genes thus far introduced — while transmitted reproductively to descendents of the host bacteria — have not been "expressed", i.e. activated; nothing to which they give rise in higher organisms has been seen in their hosts. It is increasingly speculated that it will be impossible to do more than insert them into the genome of the bacteria. Triggering their ability to produce specific proteins in their new prokaryotic host may be impossible. In this case, much of the promise of genetic engineering with bacteria might be lost — in particular the ability to persuade the bacteria to function as factories producing the products that higher organisms want and have in short supply. On the other hand, some of the danger of this new technique would also be avoided, since the bacteria have a mobility and promiscuity absent in the higher organisms which might become substitute hosts.

The failure of prokaryotes to exchange genetic material with eukaryotes in nature is believed to arise from the difference in control elements used by the cell to advise the genes when and when not to express themselves by producing the products for which they are coded. It is feared that genetic engineering might introduce into the prokaryotic world, the control methods of the eukaryotes. This, in turn, might give the prokaryotes tools that would affect the immensely intricate ecology of the world of microorganisms. Thus the Chairman of the California Institute of Technology Biology Department, Robert Sinsheimer, reports:

"Incorporation of eukaroytic DNA with its control signals into prokaryotes on an appreciable scale cannot but significantly perturb the prokaryotic-eukaryotic interaction. By recombination events these control elements could become associated with varied kinds of prokaryotic DNA. One can imagine that the viruses of prokaryotes (particularly the lysogenic species) could acquire the capacity to infect eukaryotes (consider the consequences for a eukaryotic cell of invasion by an expressible Mu-type phage — or even by a phage carrying a gene for a restriction enzyme.)"

This point of view is especially concerned because it sees any such ecological effect as irreversible; unlike aerosol cans which may be removed from the market, the replication of living things means that an escape of such organisms could not easily be undone. □

SOME TECHNICAL BACKGROUND

It is now known that the chromosomes are the carriers of heredity. The genetic material in the chromosome is called DNA (deoxyribonucleic acid). Molecules of this nucleic acid contain the genes governing specific characteristics. These genes are strung out in a linear sequence along two complementary strands of atoms, chemically bonded together, and wrapped around each other, in a helical structure known as the double helix.

The entire DNA molecule can be extremely large as molecules go; in the simplest single celled organisms such as E. Coli, the molecule can be a tenth of a centimeter long and can weigh as much as the equivalent of two billion hydrogen atoms.

Each strand of the helix is composed of a backbone of repeating groups of phosphate and sugar with four side groups (nucleotides) called bases: adenine (A), guanine (G), thymine (T) and cytosine (C). The order of the sequence of these bases determines heredity through an elegant genetic code. Thus part of the helix strand may reveal such a sequence of bases as ATGCCAGTTG. Every three letters is, in fact, an instruction to the cell to insert one of a specified set of 20 amino acids (e.g., ATG means produce the amino acid tyrosine) in a protein under construction. These twenty amino acids are, in turn, the building blocks of proteins. Hence a sufficiently long sequence of three letter codes (called codons) can instruct the cell to piece together whatever amino acids are required to construct an entire protein. Thus the DNA molecule gives instructions to build the thousands of different kinds of protein molecules which can be found in a single cell and which serve it as enzymes (elements that speed up, i.e. catalyze, chemical reactions) or as structural elements of the cell.

When a specific sequence of nucleotide bases constituting a gene for the production of some protein is to express itself, an enzyme moves along the DNA strand catalyzing chemical reactions which string together a kind of copy of the sequence in question; this translated copy called messenger RNA is a nucleic acid brother to DNA; the translation is one which simply substitutes nucleotide bases (U, C, A, G) respectively for (A, G, T, C) where U stands for Uracil. The messenger RNA carries this translation to a cell factory called a ribosome where the indicated amino acids are brought up (by transfer RNA) and bonded together by the ribosome.

What determines when the gene will or will not express itself? After all, all cells of a given organism have the same genes but in complex organisms like ourselves, the cells are performing quite different activities, hence ex-

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STANDARDS OF JUDGMENT

One important underlying reason for the existence of three different schools of thought among molecular biologists on this issue arises from their applying quite different standards for public safety.

For example, one researcher argues that the bias should be in favor of "free investigation" rather than on public dangers:

"As a working microbiologist for almost 40 years, I find it inconceivable that fear of factors inadequately known or understood should be the basis for inhibiting free investigation.

Another non-scientist observes the opposite:

"Since it is the scientists who want to do the work, let them persuade us that the experiments are safe before we permit them to continue."

Meanwhile, a senior researcher applies a very stiff standard:

"*There should be a complete stop on all forms of experimentation with artificially produced DNA recombinants that could in any way represent a potential danger to animal or plant life.*"

Perceptions of Human Mechanisms Vary Also

Not only standards of judgment are varied but also perceptions of human mechnisms. One such difference turns upon whether we can rely, to ensure proper safety regulations, upon an "invisible hand": the researcher's own fear. Noting that present experiments would produce decidedly larger quantities of hazardous material than those handled in more routine virus and virulent microorganism experiments, an observer commented:

"But there is a well-established technology for handling larger quantities of such materials and one requires assurance only that the requisite technology will be utilized. Since the laboratory workers themselves are the individuals primarily at risk, they have the greatest reason to be certain of the adequacy of the techniques to be used."

Agreeing with this, still another eminent microbiologist observed that we could depend upon pilots to determine that planes are safe; they would not fly them if they were not.

There seems obvious errors in this approach. Experience tells us that individual human beings do not take low-probability risks to themselves so seriously that they can be depended upon to protect against these risks with the conscientiousness the public desires for itself. More often, the workers become habituated to the danger and, indeed, become insensitive to it as a method of dissonance-reduction that psychologists understand quite well. Furthermore, laboratory workers (and the pilot) are motivated by temperament, training and employment to do their work and to undertake associated risks. The general public, by contrast, is primarily interested in avoiding these risks.

Anomalies in Gauging Risks

Another difference in standards arises from the attention paid to anomalies in the gauging of different risks. Molecular geneticists point out that the older microbiologists are using virulent organisms under lesser safeguards than would be required of them in analogous experiments. Turning this around, the Ashby Report observes that the older microbiologists are more careful: precautions are "second nature" to research workers familiar with the hazards of handling pathogens but "many bacterial geneticists and molecular biologists are unfamiliar with these hazards and are unable to assess the levels of precaution needed."

How new is the danger? One researcher observes that the "mere manufacture" of a recombinant DNA does not pose a hazard "per se".

"The shuffling and mixing of DNA molecules has gone on for eons and if it were dangerous to add a random piece of DNA to a plasma or virus, we would know that already. I think we must focus on those DNA's that have a potential hazard and not on the mere joining of DNA molecules."

Another adds:

"The widespread and indiscriminate use of antibacterial drugs in man and animals has exerted immeasurably more pressure on the bacterial population than could be wielded by all the research workers in this field put together."

But a third, more cautious, notes that radiation is also always with us but we have learned to seek to minimize its presence.

Degree of Risk

In the case of nuclear reactors, proponents provided estimates that suggested less than a single fatality per year per 100 reactors due to unsafe operation. Opponents drew graphs that suggested ten lives rather than one. Clearly the expected values (probability of disaster times outcome if it occurs) were thought to be quite tolerable by both sides! Obviously the public does not weigh catastrophies in proportion to their expected value outcome. Rather it considers of special concern those activities that can become major catastrophies (even if their probability is correspondingly lower). One specialist in these matters (Richard Wilson) believes that public concern rises as the square of the outcome (or even as the third power).

Maximum dangers for reactor accidents were considered to range from 1,000 fatalities (by proponents) to 50,000 (by opponents), at the level of 10^{-7} events per year. Comparably extreme events involving medical epidemics could involve another factor of 1,000 (i.e., 1,000,000 to 50,000,000 fatalities). If public concern does indeed rise as the square of the extreme catastrophe, then containment levels would have to be sharply increased beyond the seventh power of ten to a range nearer the fourteenth.

This is not now clearly understood. For example, one eminent biochemist observed that chimeric species of prokaryotic organisms that could cause disease were very unlikely, asserting:

"*Patently, no one can flatly deny such a possibility* but it is extremely remote (10^{-5}) and can certainly not be the basis for stringent regulation."

But this projected chance of one in one hundred million is not so remote by the above standard. Happily, many biochemists would argue that the overall likelihood of the creation of the dangerous strain, its escape, survival, infection and propagation is much much lower than 10^{-5} anyway.) □

INTEREST IN WHALES SOUGHT

***FAS members interested in helping research and* prepare policy statements on marine mammals should write the National Office.**

BIOLOGICAL WARFARE

No nation or sub-national group is likely to unleash a dangerous organism for which it has no vaccine and against which it has not vaccinated itself. Thus in the world of biological warfare, defense and offense are different sides of the same coin. If ,without explanation, a nation were to innoculate its entire population with a vaccine, in a future era of easy genetic engineering and in a period of international tension, who knows what alarming conclusions its neighbors might draw?

Recently, one hundred and eleven nations signed a treaty on biological warfare which has as its complete title, "Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction". The signatories (63 of which have now ratified it) undertake:

> "never in any circumstances to develop, produce, stockpile or otherwise acquire or retain:
> (1) microbial or other biological agents, or toxins whatever their origin or method of production, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes;
> (2) weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict."

The only loophole would seem to be whether a state might justify for protective purposes types and/or quantities of vaccine — justified against some purported threat —that might free it to undertake offensive action. Obviously, all this is much easier to say than to do or rely upon, but the world contains many different forces searching methods of mass destruction or coercion.

Perhaps unfortunately, the Treaty has the structure of the nuclear non-proliferation treaty in that signatories who agree to eschew military uses are assured cooperation in peaceful uses. In Article X, the states undertake to facilitate "the fullest possible exchange" of relevant equipment and information for peaceful purposes. As we are today concerned with nuclear reactors, we may someday be hoist on the same petard with biological weapons: states may sign with a view to assuring, under a peaceful guise, the information necessary to make military use of biology.

Our own military interest in this subject can be documented. Before the negotiation of the Treaty on June 9, 1969, the Defense Department's Deputy Director for Research and Engineering, Dr. D. M. MacArthur, testified that synthetic biological agents might be available within five to ten years. Dr. MacArthur emphasized the importance of developing defensive measures.

The Arms Control and Disarmament Agency has assured Dr. David Baltimore that recombinant DNA molecules do fall within the purview of the Treaty; indeed, the possibility of "altering the structure of genes" had been touched upon in the Treaty hearings before the Senate Foreign Relations Committee.

The Soviet Union also agrees with this interpretation. On February 19, 1976, Yuri Zhukov, Pravda Editor and member of the Central Committee Presidium, wrote at length about recombinant DNA. He called for the "precise and unconditional observance" of the Treaty, calling it a "document which also covers the use of genetic engineering for military purposes". But he linked his concern to General Secretary L. I. Brezhnev's call on June 13, 1975, on the prohibition of the development and production of any new types of weapons of mass destruction whatsoever. This is evidently the first indication of any concrete example of what the Soviet General Secretary had in mind. (However, the Soviet Union does not seem to be taking the problem of inadvertent spread of products of DNA recombinant experiments even as seriously as we; the Soviet Ministry of Health reports that the USSR has no limitations on this research at the naitonal, republic or institutional level.)

What can biologists do to protect against the misuse of their science for military purposes? There is a dilemma. In one sense openness is advantageous; thus the worldwide old boy network of biologists could try to keep the community informed of efforts to misuse biology for offensive purposes, and of the specific defensive methods that could protect populations against the threats being worked up. The Treaty prohibiting such offensive uses of biological warfare, signed by virtually all nations, justifies this approach. On the other hand, unrestricted openness in this evolving field could eventually put in the hands of the individual biologists power unbelievably greater than that which splitting the atom has placed in the hands of individual physicists. Only science fiction has explored problems which the next generations may now confront.

FAS asks members to write with suggestions by which the goals of the treaty on biological warfare can be advanced.

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pressing quite different genes. It is now known that repressor molecules sit on the DNA helix inhibiting enzymes from catalyzing the creation of the messenger RNA until loosened by the arrival of inducer molecules that signal the need for activity.

Viruses, Plasmids, Assorted Organelles

Genetic material is exchanged in nature not only in sexual reproduction (a process which, surprisingly, even bacteria have been shown to exhibit). Genetic material can also be transferred from one organism to another by what is called transduction. The virus, itself, nothing more than a chromosome surrounded by a protective protein coat, makes an unavoidable practice of invading cells and exploiting their far more complex machine to reproduce its chromosome and coat, hence to reproduce itself. In these cases, its progeny grow to the point of bursting the cell (and spreading the infection). Alternatively, the genetic material of the virus may become incorporated in the chromosome of the host cell, thereby transforming its future behavior and becoming perpetuated in its descendants.

A still more primitive method of exchanging genetic material occurs when different strains of bacteria exchange little bits of DNA called plasmids, which exist inside cells independently of the chromosomal DNA. In the process of transfer, they may carry along bits of the chromosome of their original cell and may even transfer it and itself into the chromosome of the second cell. It is in the area of introducing plasmid DNA into cells that recent advances have come.

Finally, it is possible to transfer genetic information by soaking cells in DNA. By a process of what is called transformation, the very delicate macromolecules of DNA can be simply absorbed in such a way that some host cells are transformed. □

BUILDING BLOCKS OF A POLICY

What follows is an effort to sketch enough of the advantages of various possible policies to provoke their serious consideration; no effort is made to provide the balanced or in-depth discussion they may deserve since we neither have the space, nor anticipate an FAS-wide consensus upon them. They are simply put forward as plausible ideas worth adding to the debate.

I. CONSTRUCTION OF REGIONAL P-4 LABORATORES: Two-thirds of the square footage of high-containment P-4 laboratories in the United States is at Fort Detrick. This leaves 175,000 square feet, of which 65,000 belongs to six laboratories in private enterprise and 110,000 square feet is divided among five universities, eight Government laboratories and 23,000 square feet at NIH.

Inevitably, projections of possible dangers will arise over the coming years before experiments have disproved them. The scientists can be expected to take whatever precautions are possible but not — so easily at least — to stop their work if precautions are not available. The existence of regional P-4 laboratories cannot help but be quite useful from time to time in persuading researchers to take greater, rather than lesser, precautions. Some non-recombinant work now done in P-3 laboratories might be usefully switched into these regional laboratories as well. And at the laboratories, run as a service to the region, one could anticipate a much more disciplined monitoring of safety procedures than one can expect in a university of colleagues. Indeed, to the extent that the critical experiments are limited to P-4 laboratories with their attendant supervision, the problem of legal controls over researchers and/or ever changing guidelines could be enormously ameliorated.

If, indeed, this research has both promise and danger, additional Government funding of P-4 laboratories would seem a simple and obvious precaution for the public to fund. (The February 28, 1975 meeting of the Recombinant Advisory Committee shows the Committee felt the priority for establishing new high risk facilities was low. But the Congress might consider it higher and might be willing to do it without requiring the funds to come out of other health budgets.)

II. LEGAL STRUCTURE FOR THE GUIDELINES: One possible way of combining the flexibility of guidelines with a legal structure that covers persons not being funded by NIH would envisage licensing the researcher. One would retain legal control over the researchers themselves while providing the possibility of continuous and abrupt change in the ukases which might be sent to them. Researchers might have to certify their knowledge of relevant safety procedures, their willingess to act in accordance with present and future guidelines, and their responsibility for what went on in this field under their supervision.

III. PROVISION FOR ALTERNATIVES TO E. COLI: Quite apart from whether experimentation is permitted to begin with E. coli, enfeebled or not, research could begin on possible substitutes in case conceptions of the risk change dramatically; we do not always want to be faced with an absence of alternatives.

IV. MOTIVATING A CONCERN: A group of senior biologists could be assigned to generate, on a continuing basis, as research proceeds, "possible danger" scenarios for their assessment by others. Most researchers see little merit in manufacturing "horror stories" and do not want to become danger-mongers. But it is for this very reason self-evidently useful to the public to have some of those biologists most informed about the subject matter explicitly charged to surface such possibilities. Such a group could be a subgroup, for example, of the DNA Advisory Committee.

V. INDUCING SAFETY RESEARCH: Safety research not being the most interesting of occupations for some of the best molecular biologists, methods must be arranged to encourage them to undertake it. Research funding could be arranged to require that a tithe of the funds allotted be devoted to safety research.

As a second suggestion, groups of laboratory workers could be retained to work inside P-4 labs under the guidance of eminent committees who, from the major universities at which they work, would oversee, by phone and visits, experimentation on delicate safety problems. A major program of safety research should also be started at NIH.

VI. AUTOMATED PROCEDURES AND MINIATURIZED EQUIPMENT: The development of procedures that could be maximally automated and miniaturized so as to lend themselves to easier containment is desirable.

VII. MONITORING THE RESEARCHERS FOR ILLNESS: Biological researchers, in general, not only those engaged in recombinant DNA experiments, should be watched more closely than they are, and followup studies made, to protect the researchers and to provide an early warning of possible problems the public might face.

VIII. CLONE BANK: One of the most controversial experiments results when the entire DNA of a cell is divided up into segments of the approximate length of a gene and each gene placed inside bacterium which multiplies into a clone. The hazard lies in the possibility that dangerous genes along the DNA segment will have been multiplied in the process. It has been suggested that such "shot gun" experiments could be restricted to P-4 laboratories and the results made available to researchers in the following way.

After the separate genes are emplanted in separate bacteria and clones developed, the bacteria would be broken open and the resultant cloned DNA left as a residue could be isolated. Persons interested in working with the cloned DNA could have it sent to them from the Clone Bank. Inasmuch as each researcher would have to determine which was the piece of DNA that corresponded with the gene of interest to him, the entire set of clone-derived DNA's would be sent to him. But since the genetic material is being delivered to him as purified DNA rather than inside different bacteria, it is much safer to handle. The naked DNA is far less likely to be absorbed by a cell than is a bacterium carrying the DNA to infect some organism or to exchange genetic material with one.) Thus a clone bank would permit the most dangerous stage of dealing with unknown genes to be done at rare intervals, with maximum containment, rather than have it become an every day occurrence.

IX. PUBLIC HEALTH SECTOR: It should not be overlooked that the sector of public health specialists forms a second line of defense against escaped chimeric organisms and may deserve special funding.

THE GUIDELINES IN BRIEF

Four methods of physical containment are specified as follows:

P1 (minimal): strict adherence to standard practices;
P2 (low): limited access to laboratory during experiments; precautions against the release of aerosols and the prohibition of mouth pipetting;
P3 (moderate): laboratories equipped to ensure inward air flow; biological safety cabinets; wearing of gloves; decontamination of recirculated air; (alternative procedures suggested when air conditions cannot be controlled as specified);
P4 (high): special facilities of the kind used in biological warfare designed installations, such as isolation of airlocks, clothing changes and showers, decontamination of all air, liquid and solid wastes.

Three levels of biological containment were suggested when using the K-12 strain of Escherichia Coli (E. Coli) as a host for recombinant DNA molecules:

EK1: use of E. Coli K-12 in its usual form;
EK2: use of modified E. Coli K-12 hosts and modified E. Coli hosts and modified plasmid or bacteriophage "vectors" so that the survival rate of the recombined chimera is less than one in 10^6 in the natural environment, i.e. if the host escapes the laboratory;
EK3: use of EK2 systems for which the increased containment has been independently confirmed by animal tests.

Experiments To Be Prohibited At This Time

1. Experiments taking their DNA from highly pathogenic organisms.
2. Experiments in which the DNA to be joined contains genes for production of highly toxic agents.
3. Experiments in which the DNA is derived from a plant pathogen if the host may acquire increased virulence or range.
4. Experiments involving uncontrolled release of organisms containing recombinant molecules.
5. Transfer of genes conferring drug resistance to microorganisms not known to acquire such resistance naturally, when the resistance may compromise clinical use of the drug in medicine or agriculture.
6. Large scale experiments with recombinant DNAs known to result in the formation of harmful products (with exceptions possible if approved by the NIH Recombinant DNA Advisory Committee).

Classification of Permitted E. Coli Experiments

EK2 modified strains of E. Coli must be used if the foreign DNA to be inserted into E. Coli comes from the chromosomes of vertebrates or higher plants; and, in particular, when it comes from mammals or birds, P3 containment is required. (P4 for primates.)

If the foreign DNA comes from the chromosomes of prokaryotes known to exchange genetic material with E. Coli in nature, then the enfeebled EK2 strains are required only if the prokaryote is considered a moderate known hazard.

If the prokaryotes do not exchange genetic information with E. Coli, enfeebled strains may or may not be required depending upon details.

Clones (colonies descended from single cells) arising from the DNA recombinant host can be used under normal laboratory conditions once it is established that the originating chimera is free of harmful genes.

If the foreign DNA is derived not from chromosomes but from viruses that infect animals, EK2 or EK3 strains are to be used with P4 or P3 levels of containment. (However, if it comes from viruses that infect plants, normal strains can be used with P3 containment.)

If the foreign DNA is derived from purified eukaryotic organelle DNA, ordinary strains can be used of E. Coli with P3 containment (this includes primates).

If the foreign DNA arises from prokaryotic plasmids or bacteriophages, then modified EK2 strains are required only if the prokaryote is known as a moderate hazard.

Animal Host Cells

When animal cells are used as the host in tissue culture experiments, the problem of escape becomes one of containing the "vector" used to carry the recombinant molecule into the animal cell. Two animal viruses considered sufficiently well-studied at present for such use are polyoma virus (which infects mice) and simian virus 40 (SV 40) (which infects monkeys). Both cause tumors in mammals and SV40 infects humans. The guidelines suggest using polyoma DNA defective in its ability to infect and reproduce and asks P3 containment (P4 if the foreign DNA to be placed in the vector comes from pathogenic organisms designated Class 2 by the Center for Disease Control — Class 3, 4 and 5 organisms are prohibited entirely).

For SV40, the guidelines require defective SV40 and P4 conditions which can, however, be weakened to P3 if no infectious viral particles are produced and if specified criteria for purity and lack of harmful genes have been met.

THE TROUBLE WITH E. COLI

According to a report by Dr. Stanley Falkow, there are an almost astronomical number of distinct strains of Escherichia Coli varying by as much as 25% in their nucleotide seqeuence of bases. Some strains cause infantile diarrhea or diseases resembling bacillary dysentary or "traveller's diarrhea" in adults in developing countries. E. Coli is not ordinarily a highly virulent organism but it may acquire accessory genetic information, usually through plasmids, to tip the balance between irrelevant co-habitation and the ability to initiate overt disease. He concludes:

> "From the standpoint of recombinant DNA molecules, the documentation of the effects of plasmid-mediated determinants on pathogenicity must be reviewed as one of the most cogent arguments for the potential biohazards associated with this research."

E. Coli are disseminated in many ways (e.g. have many vectors in nature) and plasmids and bacterial viruses are very common in them. With specific reference to the strain K-12 normally under discussion, Dr. Folkow observes:

> "It is also clear from our studies that a carried plasmid may have a profound effect on the survival and carriage of E. Coli K-12 . . . it may not be too far-fetched to suggest that some DNA recombinant molecules could profoundly affect the ability of this E. Coli strain to survive and multiply in the gastrointestinal tract."

E. Coli has been characterized as being of "untiring promiscuity", exchanging genetic material with a variety of organisms and transferring its plasmids to other cells.

1976 OFFICERS NOMINATED

Candidates for Chairman:

George William Rathjens: Ph.D. Chemistry, 1951, now Professor of Political Science at MIT. Formerly: staff of the Office of Science and Technology in the Arms Control and Disarmament Agency (ACDA); Director Weapons Systems Evaluation Division (WSED) in the Institute for Defense Analyses (IDA). Dr. Rathjens is a long-time FAS official and an active leading participant in its arms control activities. He played a major role in the opposition to the Anti-ballistic missile and testified and worked against the Supersonic Transport as well. He is one of America's most experienced systems analysts on high technology military and civilian systems and, among other things, is currently studying nuclear reactors on a number of state and national committees.

Frank Von Hippel: Center for Environmental Studies, *Engineering School, Princeton University.* D. Phil. Oxford University, 1962. Assistant Professor of Physics, Stanford University. Sloan Foundation Fellow. Formerly member, Staff Theory Group, High Energy Physics Division, Argonne National Laboratory; Resident Fellow NAS; Editorial Board, *Bulletin of the Atomic Scientists.* Dr. Von Hippel is the author, with Joel Primack, of a recent widely-read book on science and public policy entitled Advice and Dissent". He is playing a major role in the debate over nuclear power. He was an organizer and participant in the American Physical Society reactor study, and more recently, has played a leading role in persuading the AAAS to work on problems of freedom for scientists. He is now engaged full-time working on independent nuclear energy policy studies.

Candidates for Council

Lipman Bers: President of the American Mathematical Society (AMS); Chairman, Department of Mathematics, Columbia University; former Chairman: Division of Mathematical Sciences of the National Research Council; Mathematics Section of AAAS. Professor Bers has been extremely active and influential in the defense of scientists abroad in such diverse areas as the Soviet Union and Uruguay.

Geoffrey Chew: Chairman, Department of Physics, University of California, Berkeley. A research assistant at the Los Alamos bomb project in the fifties, he has been awarded the Ernest O. Lawrence Memorial award and the APS Hughes prize. Author of "A Basis for Nuclear Democracy", Dr. Chew is a long-time FAS member who, in particular, chaired an FAS committee on rights to travel and passports from 1952-1960.

Myra Karstadt: Ph.D. Biochemistry, 1969, on biochemistry of nucleic acids. Dr. Karstadt went on to do postdoctoral work in environmental health sciences and to receiving a law degree (Harvard, 1972). She served on the staff of the Office of Technology Assessment (OTA) and at the Environmental Law Institute. Currently a consultant to the National Institute of Environmental Health Sciences, she has advised FAS on a number of topics in environmental law and toxic chemicals.

Laurence I. Moss: M. S. in Nuclear Engineering; engineer and consultant; former Executive Secretary of the Committee on Public Engineering Policy, National Academy of Engineering; White House Fellow, '68-'69; former President, Sierra Club; organized Coalition Against the SST and the Coalition to Tax Pollution. A former member of the FAS Council, Mr. Moss has written widely and influentially on environmental matters of all kinds.

Franklin A. Neva: Chief of Laboratory of Parasitic Diseases, NIH; formerly a member of the Harvard School of Public Health; and a holder of the Bailey K. Ashford award of the American Society of Tropical Medicine. Dr. Neva is an FAS Sponsor.

David Robinson: Vice President of the Carnegie Corporation of New York, Dr. Robinson received a Ph.D. in Chemical Physics and later served as a scientific liaison officer in the Office of Naval Research. From 1961-67 he was senior staff member of the Office of Science and Technology (OST) in the White House. He has been consultant to the President's Science Advisory Committee (on health care and educational technology); to NSF; to NAS (on R&D, minorities in science and women in science); and to New York State (on nuclear energy and of engineering education.) A long-time FAS member.

William Shurcliff: Senior Research Associate, Cambridge Electron Accelerator, Harvard University; Technical Historian of First Atomic Bomb Tests at Bikini; author of "Bombs at Bikini", "Polarized Light". As Director and organizer of the Citizen's League Against the Sonic Bomb in 1967, he created that grassroots lobby against the SST and worked indefatigably from his home to maintain it. Dr. Shurcliff is now engaged in a similar effort to encourage the local use of solar power.

Alvin Weinberg: Director of the Institute for Energy Analysis; during 1974, Director of the U.S. Federal Energy Administration (FEA) Office of Energy Research and Development; previously, for more than a quarter century, Director of the Oak Ridge National Laboratory. Dr. Weinberg helped design the first nuclear reactors, wrote the definitive work in this field ("Physical Theory of Neutron Chain Reactors", with Eugene Wigner) and has received the Atoms for Peace award and the E. O. Lawrence Memorial award. He has written eloquently and widely on science and public policy matters ("Reflections on Big Science", April, 1967, is a book length example) and his pungent and incisive phrases have been adopted by both supporters and opponents of his conclusions.

Robert H. Williams: Research Scientist, Center for Environmental Studies, Princeton University; Ph.D. in Theoretical Physics, 1967; formerly Director of Research, Institute for Public Policy Alternatives, SUNY, Albany. Dr. Williams was a senior scientist of the Ford Foundation Energy Policy Project and a chief architect and draftsman of several of its component parts. He specializes in energy policy with special emphasis on solar power, nuclear power and conservation.

Preliminay Straw Ballot on Guidelines

I am _____ am not _____ a biologist.

On the basis of the April Report and other general reading, I lean toward the school of thought that believes the guidelines are: A. Probably too restrictive ☐ B. Probably about right ☐ C. Probably insufficiently cautious ☐ D. Insufficient information ☐

FEDERATION OF AMERICAN SCIENTISTS — 1976 BALLOT

Nine candidates appear for the six Council positions in accordance with the requirement that at least 50% more candidates stand for election than there are positions available. **ALL BALLOTS MUST BE MARKED FOR AT LEAST THREE COUNCIL CANDIDATES, AND NO MORE THAN SIX.**

Chairman

- ☐ Rathjens
- ☐ Von Hippel

Council Delegates

(Vote for at least three).

- ☐ Bers
- ☐ Chew
- ☐ Karstadt
- ☐ Moss
- ☐ Neva
- ☐ Robinson
- ☐ Shurcliff
- ☐ Weinberg
- ☐ Williams

Your vote cannot be counted unless you are a member whose dues have been paid for calendar year 1976. All members have been billed ... once in the fall and once in February. If you have not renewed thus far, you may do so with your ballot.

Along with the election ballot, it is our custom to stimulate criticisms, comments and suggestions about the last year's activities and newsletters, as well as proposals for the coming one's. (The May issue will cover "Cancer and the Environment" and the June issue is scheduled for National Security Policy.) Send these in separate letters or notes with your name and address; you may attach them, however, to this ballot.

NOW, TEAR THIS PAGE OUT OF YOUR NEWSLETTER, SIGN YOUR NAME BELOW IN THE INDICATED PLACE, CHECK OFF YOUR PREFERENCE FOR CHAIRMAN. CHECK AT LEAST THREE OF THE CANDIDATES FOR COUNCIL MEMBERS, THEN FOLD THIS PAGE, TAPE OR STAPLE IT CLOSED, PUT A STAMP ON IT, AND MAIL IT TO US. ALL BALLOTS MUST ARRIVE HERE BY MAY 15 TO BE COUNTED.

BALLOT WITHIN

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_____

(Signature of Voter)

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☐ I wish to join FAS and receive the newsletter as a full member.
Enclosed is my check for 1976 calendar year dues. (☐ I am not a natural or social scientist, lawyer, doctor or engineer, but wish to become a non-voting associate member.)

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☐ *FAS Public Interest Report — $20 for calendar year*

☐ Enclosed is my tax deductible contribution of _____ to the FAS Fund.

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STAPLE OR TAPE THIS CLOSED AFTER FOLDING

IN DEFENSE OF PLASMID ENGINEERING

In reviewing the literature on recombinant DNA we found nothing (of less than impenetrable technicality) that attempted the difficult task of sketching for general biological publication why none of various alarming speculations would come to pass. Finding it disturbing that so many scientists would hew to sanguine convictions, however well-founded, without subjecting them to a test of scrutiny by biologists as a whole at least, we challenged one laboratory group to commit its sense of the hazards to paper in a reasonably readible form.

What follows is such an effort, footnoted by referee criticisms. Its authors declined to indicate its authorship for a number of reasons including the time pressures under which it necessarily was prepared. We put it forward, nevertheless, as the best such document we know of its kind in order to catalyze comments from our more informed readers, in support or opposition; these we will survey in the June Public Interest Report. *For the reader who is not in biology, it provides a glimpse, that may be educational in itself, of the relevant complexities.*

We believe that the concern expressed from some sectors over the alleged hazards of experiments involving recombinant DNA, while well-intentioned, has little if any realistic basis. The few scenarios proposed in which the introduction of eukaryotic DNA results in harm to man, or in an alteration of bacterial ecology, would require the coordinated operation of so many highly improbable mechanisms, that the overall likelihood of their occurrence is negligible.

Purported hazards involve either recombination between eukaryotic DNA in *E. coli* and the human genome, with ensuing deleterious effects on the human host; expression of eukaryotic DNA in *E. coli,* producing a substance harmful to the human host; insertion into *E. coli* of an eukaryotic DNA fragment capable of "benefically" modifying the bacterium, producing a "super-bacterium", and thereby upsetting microbial ecology; or cloning of DNA sequences from oncogenic viruses, and subsequent expression of the oncogenic viral genes in *E. coli,* or transfer into human cells.

The following considerations argue against the possibility of any of these events. In order for an eukaryotic DNA sequence in a plasmid to recombine with human DNA, it would first have to enter a human cell, either by transfer from a bacterium or by uptake of the DNA by a cell. Transfer of DNA from bacteria to human cells is not known to occur, and if it does it is certainly a rare event. [1] If direct uptake of DNA by human cells does occur, which is also uncertain, eukaryotic DNA fragments in bacterial plasmids are probably no more ineffective than the eukaryotic DNA itself. [2] Yet thousands of scientists have handled large amounts of eukaryotic DNA, with no special precautions, for many years, and no ill effects have been observed. If a eukaryotic DNA fragment could get into a human cell, what are the chances that it would recombine with the human genome? It would have to survive the nucleolytic enzymes which exist in the cell, and find its way into the nucleus. Also, the genomes of non-dividing, somatic cells might be incapable of engaging in recombination. [3] A DNA fragment from any subprimate eukaryote would be highly unlikely to recombine with the human genome, even if cellular conditions permitted recombination, because subprimate DNA has very little or no sequence homology with human DNA, while recombination is considered to require regions of sequence homology. [4] From this point of view, shotgun experiments with primate DNA are not as hazard-free as those with subprimate DNA. If an eukaryotic DNA fragment did manage to recombine with the genome of a human somatic cell, would it be expressed in a way deleterious to the cell? This would require 1) that it be inserted at a site where it would interfere with an essential cell function; or 2) that it be inserted next to a control region, causing it to be transcribed. Furthermore the transcript would have to contain translational initiation and termination signals which would be correctly interpreted by the human cell, and the product would have to be harmful to the cell. Any deleterious effect to a single cell, furthermore, would probably be inconsequential for the whole organism. [5] Multiplication of the extremely low probabilities of each of these steps shows that the overall scenario is simply implausible.

In order for *E. coli* to produce a toxic substance from an eukaryotic DNA, the fragment must first code for a toxic polypetide. This is probably an extremely rare event in itself. Next, the fragment must be correctly transcribed, and the transcript must accumulate in sufficient amounts and be translated correctly and efficiently. *E. coli* RNA polymerase, however, does not specifically recognize eukaryotic transcriptional initiation and termination sites, so transcription of eukaryotic genes is likely to be incomplete and inefficient in *E. coli.* [6] For example, *E. coli* carrying a plasmid containing sea urchin histone genes makes no detectable histone, though histone messenger RNA sequences are transcribed. This indicates that either the transcripts are incomplete, or faithful translation of the message does not occur. Furthermore, if a toxic protein were produced in *E. coli,* it would have to be released by the bacteria in sufficient amounts in order to be harmful. Many toxic proteins might be toxic to the bacteria themselves, so that plasmids carrying the genes for such toxins could never be cloned. [7] In short, the production of a toxic bacterium would also require that many highly improbable events occur in concert. This is not to say that such an event could not be deliberately engineered after many years of laboratory work.

Similar objections apply to the suggestion that introduction of an eukaryotic DNA fragment might increase the viaibility of *E. coli* to the detriment of normal bacterial ecology. Expression of an eukaryotic gene in *E. coli* is unlikely for reasons already described. [8] If an eukaryotic gene were expressed, it would be much more likely to interfere with bacterial functions, or simply have no

—Continued on page 12

INFORMAL GLOSSARY FOR THE NON-BIOLOGIST: *Eukaryote:* organism whose cells have a nucleus as do higher organisms; *Prokaryote:* organisms whose cells have no nucleus as in the case with bacteria; *Clone:* set of cells derived from a single parental cell through successive divisions; *Plasmid:* extra-chromosomal genetic element; *Somatic cell:* non-sex cell; *Oncogenic:* tumor-inducing; *Sequence Homology:* degree of sequence correlation; *Polypeptide:* chain of amino acids; *RNA Polymerase:* the catalyst which transcribes an RNA copy from the DNA; *Genome:* total genetic material.

Continued from page 11

effect, than to favorably alter the bacterium. The genes for any simple metabolic enzymes that might be useful to the bacterium have certainly been available during the billion or more years of its evolution, throughout which it has had ample opportunity for recombination. This statement can also be made for any animal DNA sequences, to which *E. coli* have also had prolonged access during their evolution. In any case eukaryotic DNA sequences such as control sequences could probably not function in a bacterial genome. The idea that an eukaryotic DNA fragment could endow a bacterium with novel properties by constructively altering bacterial regulatory mechanisms severely underestimates the complexity of genetic regulation, and is thus very naive.

Even the cloning of purified oncogenic viral DNA in *E. coli* may not be as hazardous as is commonly assumed. Animal virus gene expression requires the molecular machinery of eukaryotic host cells and is most unlikely to occur correctly in *E. coli*, particularly if only viral DNA restriction fragments, with only a fraction of the viral genome, are cloned. Viral DNA restriction fragments would seem far less capable of infecting human cells when incorporated in bacterial plasmids than is the complete virion which includes an efficient infective coat and all the genetic information required for viral assembly. Whatever the danger in cloning viral DNA, it is probably a much safer way to study the structure of viral genomes than handling the oncogenic viruses themselves. Since it is clearly desirable that the nature of oncogenic viruses be investigated, the cloning of viral restriction fragments in *E. coli* is not entirely lacking in justification. The carefully controlled examination of special fractions of the viral genome is to be compared with the long history of random and uncontrolled introductions of pathogens into a variety of hosts over many years in clinical research.

While it is extremely improbable that cloning eukaryotic DNA fragments in *E. coli* plasmids is actually hazardous, the benefits of such experiments for our understanding of the eukaryotic genome are real and immediate. This technology permits the isolation and characterization of specific regions of the genome, which is tremendously difficult, if not impossible to accomplish by other means. It is currently being used in several laboratories to study the DNA sequences in the vicinity of animal structural genes. These sequences are believed to be involved in controlling the expression of the structural genes, and their characterization is essential for our understanding of animal gene regulation. An understanding of many other aspects of genome organization and function is also within reach for the first time through the use of recombinant DNA techniques. Since progress in our understanding of eukaryotic genome function is clearly prerequisite to an understanding of the nature of living systems, as well as of cancer, and many other genetic and developmental disorders, to delay these experiments on the basis of highly improbable hazards is unconscionable.

REDRESSING REFEREE COMMENTS:

1) Transfer of DNA from bacteria to human cells could easily occur in such cases as those in which Salmonella colonizes human cells and dies; and it is not certain that the transfer of DNA is a rare event.
2) DNA fragments in bacterial plasmids may be more infective than the eukaryotic DNA itself since the plasmids are designed by nature for exchange of genetic material and are less fragile than linear eukaryotic DNA fragments.
3) The argument here is weak because the nucleoytic enzymes are not an insurmountable barrier to survival of the DNA and because most body cells are not strictly "non-dividing".
4) Recombination does not always require regions of sequence homolgy.
5) While it may be true that deleterious effects to a single cell would "probably" be inconsequential for the whole organism, the most serious cases at issue — the ones in which cancer may be involved — are ones in which a single cell could lead to the destruction of the organism.
6) It is not so clear that this transcription is "likely to be incomplete and inefficient".
7) What is a toxic protein for man and for bacteria are quite different things and it is too strong to suggest that "many" proteins toxic to man would be incapable of being cloned to a bacteria.
8) The twice repeated argument that expression of eukaryotic genes in *E. Coli* is "unlikely" is still being fought out in experimental study and is being dismissed too lightly. □

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