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Summary Statement of the Asilomar

Conference on Recombinant DNA Molecules

I. Introduction and General Conclusions

This meeting was organized to review scientific progress in the research on recombinant DNA molecules and to discuss appropriate ways to deal with the potential biohazards of this work. We have heard about the impressive achievements already made in this field and seen glimpses of the remarkable potential of these methods for furthering our understanding of the fundamental processes in eukaryote cells. The use of recombinant DNA methodology promises to revolutionize the practice of molecular biology. While there has as yet been no practical application of the new techniques, there is every reason to believe that they will have significant impact in the future.

Of particular concern to the participants of the meeting was the issue of whether the pause in research in this area, called for in the letter published in July, 1974, by the Committee on Recombinant DNA Molecules of the National Academy of Sciences, U.S.A., should end; and, if so, whether there are ways in which the scientific work can be undertaken with minimal risks to workers in laboratories, to the public at large and to the animal and plant species sharing our ecosystems. It was emphasized that, in the longer term, even more difficult problems may arise in the large scale application of this

work in industry, medicine and agriculture. Even in the present, more limited conduct of research in this field, the evaluation of potential biohazards has proved to be extremely difficult. The new techniques permitting combination of genetic information from very different organisms place us in an area of biology with many unknowns. It is this ignorance that has compelled us to conclude that it would be wise to exercise the utmost caution. Nevertheless, it was agreed that most of the work on construction of recombinant DNA molecules should proceed but with appropriate safeguards, principally, biologic and physical barriers adequate to contain the newly created organisms. Further, the participants of the Conference concluded that there are certain experiments in which the potential risks are of such a serious nature that they ought not to be done with presently available containment facilities. Future research and experience may show that many of the potential biohazards are less serious and less probable than we now suspect. However, it was agreed that standards of protection should be greater at the beginning and modified as assessments of the risks change. Moreover, an anticipated change in experimental design causing increased risks, however small, should be carefully evaluated.

II. Principles Guiding the Recommendations and Conclusions

Though our assessments of the risks involved with each of the various lines of research on recombinant DNA molecules may differ, few, if any, believe that this methodology is free from any risk. Reasonable principles for dealing with these potential risks are: 1) that containment be made a significant consideration of the experimental design and; 2) that the effectiveness of the containment should match as closely as possible the estimated risk. Consequently, whatever scale of risks is agreed upon, be it a three, four or multi-category classification, there should be a commensurate scale of containment. Estimating the risks will be difficult and empirical at first but this will improve as we acquire additional knowledge; at each state we shall have to match the potential risk with an appropriate level of containment. Experiments requiring large scale operations would seem to be riskier than equivalent experiments done on a small scale and, therefore, require more stringent containment procedures. Similarly the use of cloning vehicles or vectors (plasmids, phages) and bacterial hosts with a restricted capacity to multiply outside of the laboratory would increase the safety of a particular experiment. The ways in which potential biohazards and different levels of containment are matched may vary from time to time particularly as the containment technology is improved. Quite likely the means for assessing and balancing risks with appropriate levels of containment will need to

be reexamined from time to time. Hopefully, through both formal and informal channels of information within and between the nations of the world, the way in which potential biohazards and levels of containment are matched would be consistent.

Containment of potentially biohazardous agents can be achieved in several ways. The most important, because it contributes most significantly to limiting the spread of the recombinant DNAs, is the use of biological barriers. These barriers are of two types: 1) Fastidious bacterial hosts unable to survive in natural environments, and 2) non-transmissible and equally fastidious vectors (plasmids, bacteriophages or other viruses) able to grow only in specified hosts. Physical containment, exemplified by the use of suitable hoods, or, where applicable, limited access or negative pressure laboratories, provides an additional factor of safety. Additionally, adherence to good microbiological practices which to a large measure can limit the escape of organisms from the experimental situation, contributes measurably to the safety of the operation. Consequently, education and training of all personnel involved in the experiments is essential to the effectiveness of all containment measures. In practice the different means of containment will complement one another and substantial improvements in the ability to restrict the growth of bacterial hosts and vectors could permit modifications of the complementary containment requirements.

Stringent physical containment and rigorous laboratory procedures can reduce but not eliminate the probability of spreading potentially hazardous agents. Therefore, investigators relying upon "disarmed" bacterial hosts and vectors for additional safety must test rigorously the effectiveness of these agents before accepting their validity as biological barriers.

III. Specific Recommendations for Matching Types of Containment with Types of Experiments

No classification of experiments as to risk and no set of containment procedures can anticipate all situations. Given our present uncertainties about the hazards, the parameters proposed here are broadly conceived and meant to provide provisional guidelines for investigators and agencies concerned with research on recombinant DNAs. But each investigator bears a responsibility for determining whether, in their particular case, special circumstances warrant greater care than is suggested here.

A. Types of Containment

1. Minimal Risk: This type of containment is applicable to experiments in which the biohazards may be reasonably assessed and are expected to be minimal. Such containment can be achieved by following the operating procedures recommended for clinical microbiological laboratories. Essential features of such facilities are a ban on drinking, eating or smoking in the laboratory, wearing laboratory coats in the work area, the use of cotton-plugged pipettes or preferably mechanical pipetting devices and prompt disinfection of contaminated materials.

Baltimore-Roblin version of 'low risk' made a positive statement about use of 'controllable' agents. This rewrite puts change in controllability as a variable possibly arising from the experiment per se and then only restricts if a change to ineffective treatment is unlikely. I like the original much better. This is too loose at present. (Who knows what 'unlikely' means!)

2. Low Risk: This level of containment is appropriate for experiments which generate novel biotypes but where the available information makes it unlikely that the recombinant DNA can alter appreciably the host's ecological behavior, increase significantly its pathogenicity, or prevent effective treatment of any resulting infections. The key features of this containment (in addition to the minimal procedures mentioned above) are a ban on mouth pipetting, access limited to laboratory personnel, and the use of biological safety cabinets for procedures likely to produce aerosols (e.g., blending and sonication).

Our original added to 'low' the statement: While existing vectors can be used for this level of containment, as safer vectors become available, their use is recommended.' I would like to see this put back.

3. Moderate Risk: Such containment facilities are for experiments in which there is a probability of generating an agent with ~~a~~ significantly ~~greater~~ potential for pathogenicity or ecological disruption. The principle features of this level of containment, in addition to those of the two preceding classes, are that transfer operations should be carried out in biological safety cabinets (e.g., laminar flow hoods), gloves should be worn during the handling of infectious materials, vacuum lines and drains must be protected by filters and negative pressure should be maintained in the limited access laboratories. Moreover, such experiments must be done only with vectors and hosts that have an appreciably reduced capacity to multiply outside of the laboratory.

greater than what? omit greater. Redo as indicated which essentially returns to B-R wording

4. High Risk: This level of containment is for experiments in which the effects on the ecological potential or pathogenicity of the modified organism could be severe and thereby pose a serious biohazard to the investigator or the public at large. The main features of this facility, which was designed to contain highly infectious microbiological agents, are its isolation from other areas by air locks, a negative pressure environment, a requirement for clothing changes and showers for entering personnel and laboratories fitted with treatment systems to inactivate or remove biological agents that may be contaminants in exhaust air, liquid and solid wastes. All persons occupying these areas should wear protective laboratory clothing and shower at each exit from the containment facility. The handling of agents should be confined to biological safety cabinets in which the exhaust air is incinerated or passed through Hepa filters. High risk containment includes, beside the physical and procedural features described above, the use of rigorously tested vectors and hosts whose growth can be confined to the laboratory.

B. Types of Experiments

Accurate estimates of the risks associated with different types of experiments are difficult to obtain because of our ignorance of the probability that the anticipated dangers will manifest themselves. Nevertheless, experiments involving the construction and propagation of recombinant DNA molecules using DNAs from 1) prokaryotes, bacteriophages and other episomes, 2) animal viruses, and 3) eukaryotes have been characterized as minimal, low,

moderate and high risks to guide investigators in their choice of the appropriate containment. These designations should be viewed as interim assignments which will need to be revised upward or downward by future experience.

The recombinant DNA molecules themselves, as distinct from cells carrying them, may be infectious to bacteria or higher organisms. DNA preparations from these experiments, particularly in large quantities, should be chemically inactivated before disposal.

I prefer Berg's more-positive sentence on p2 of their paper, with the sentence following this one.

1. Prokaryotes, bacteriophages and other episomes: Where the construction of recombinant DNA molecules and their propagation involves prokaryotic agents that can normally exchange genetic information, the experiments can be performed in minimal risk containment facilities. Low risk containment facilities should be used where the elements comprising the recombinant DNA molecules are derived from agents that ordinarily do not exchange genetic information. If the experiments involve genetic determinants from pathogenic organisms or the creation of organisms with increased pathogenicity for man or the environment, a moderate or high risk containment should be used depending upon the severity of the potential hazard. Experiments extending the range of antibiotic resistance of the recipient species or otherwise compromising the controllability of known pathogens should be undertaken only under moderate or high risk containment depending upon the virulence of the organism involved.

2. Animal Viruses: Experiments involving linkage of viral genomes to prokaryotic vectors and their propagation in prokaryotic cells should be performed only with moderate risk containment capability. Rigorously purified and characterized segments of non-oncogenic viral genomes or of the demonstrably non-transforming regions of oncogenic viral DNAs can be attached to existing vectors and propagated in E. coli using moderate containment facilities.

E. coli is ambiguous. "Safe" E coli? presumably we mean any E. coli since otherwise this group is not differentiated from group in 1st sentence

Experiments designed to introduce or propagate DNA from non-viral or other low risk agents into animal cells should use only low risk animal virus DNAs as vectors and manipulations should be confined to moderate risk containment facilities.

3. Eukaryotes: The risks associated with joining random fragments of eukaryote DNA to prokaryotic DNA vectors and the propagation of these recombinant DNAs in prokaryotic hosts are the most difficult to assess.

A priori, the DNA from warm-blooded vertebrates is more likely to contain cryptic viral genomes potentially pathogenic for man than is the DNA from other eukaryotes. Consequently attempts to clone segments of DNA from such animal genomes should be performed in a moderate risk containment facility with the safest vector-host system available. Until cloned segments of warm-blooded vertebrate DNA are completely characterized, they should continue to be maintained in the safest vector-host system in moderate risk containment laboratories.

This means OK to do now with existing hosts & vectors. Consider the experiment ō primate DNA. Do we really think this should be done without good biological barriers?

Unless the organism makes a product known to be dangerous (e.g., toxin, virus), recombinant DNAs from cold-blooded vertebrates and all other lower eukaryotes can be constructed and propagated with the safest vector-host system available in low risk containment facilities.

Purified DNA from any source that performs known functions and can be judged to be non-toxic, may be cloned with currently available vectors in low risk containment facilities. (Toxic here includes potentially oncogenic products or substances that might perturb normal metabolism if produced in an animal or plant by a resident microorganism.)

4. Experiments To Be Deferred: There are feasible experiments which present dangers serious enough that their performance at this time should not be undertaken with the presently available physical and biological containment capability, ~~and currently available vector-host systems~~. These include the cloning of recombinant DNAs derived from highly pathogenic organisms, DNA containing toxin genes and very large scale experiments using recombinant DNAs that are able to make products potentially harmful to man, animals or plants.

You deleted paragraph about large scale. O.K with me since it was ambiguous, and idea is expressed ~~elsewhere~~. better here and elsewhere

IV. Implementation

In many countries steps are already being taken by national bodies to formulate codes of practice for the conduct of experiments with known or potential biohazard. Until these are established, we urge individual scientists to use the proposals in this document as a guide. In addition, there are some recommendations which could be immediately and directly implemented by the scientific community.

A. Development of Safer Vectors and Hosts

One of the most important and interesting accomplishments of the meeting was the realization that special bacteria and vectors can be designed and constructed and that the use of these could radically enhance the safety of experiments by many orders of magnitude. Appendix G contains a summary of the suggestions; it is certain that in the near future there will become available variants of λ bacteriophage, non-transmissible plasmids and special strains of *E. coli* all of which could, in principle, reduce biohazards by very large factors, as well as bringing about technical improvements in the methods themselves. Other bacteria, particularly mutably modified strains of *B. subtilis*, may also be specially useful for particular purposes. There is also the possibility that a suitable vector may be found for simple eukaryotic cells such as yeast. There should be a continuous development in this area and the participants at the meeting agreed that all such strains will be made freely available.

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B. Laboratory Procedures

It is the clear responsibility of the principal investigator to inform the staff of the laboratory of the hazards of such experiments, before they are initiated. Free and open discussion is necessary so that each individual fully understands the nature of the experiment and any risk that might be involved. ~~This is particularly true for~~ All personnel ~~such as~~ including technicians and non-professional staff should be included. ~~who may not have "authorship" status.~~ All workers must be properly trained in the containment procedures that are designed to control the hazard, including the emergency action in the event of a hazard. It is also recommended that appropriate health surveillance of all personnel, including serological monitoring, be conducted periodically.

find this wording not to my liking.

① why 'particularly'?
② suggest rewrite inserted

C. Education and Reassessment

Research in this area will develop very quickly and the methods will be applied to many different biological problems. It is impossible at any given time to foresee the entire range of all potential experiments and make judgments on them. It is therefore essential to undertake a continuing reassessment of the problems in the light of new scientific knowledge. This could be achieved by a series of annual workshops and meetings, some of which should be at the international level. There should also be courses to train individuals in the relevant methods since it is likely that the work will be taken up by laboratories which may not have had extensive experience in this area. Consideration should also be given to the establishment of new information pertinent to the effectiveness of biological containment.

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V. New Knowledge

This document represents our first assessment of the potential biohazards in the light of current knowledge. Very little is known about the survival of laboratory strains of bacteria and bacteriophages in different ecological niches in the outside world. Even less is known about the possibility of recombinant DNA molecules to enhance or depress the selective value of their vectors in Nature. These questions are fundamental to the testing of any new organisms that may be constructed. Research in this area will have to be undertaken and should be given high priority. The difficulty is that, in general, molecular biologists who may construct DNA recombinant molecules do not undertake these experiments and it may be necessary to facilitate collaborative research between them and groups skilled in the study of bacterial infection or ecological microbiology. Such work should also result in methods to monitor any possible escape or dissemination of cloning vehicles.

Nothing is known about the potential infectivity in higher organisms of phages or bacteria containing segments of eukaryotic DNA and very little about the infectivity of the DNA molecules themselves. Genetic transformation of bacteria does occur in animals suggesting that DNA molecules can retain their biological potency in this environment. There are a large number of questions in this area the answers to which are essential for our assessment of the biohazards of experiments with recombinant molecules. It will be necessary to ensure that this work will be planned and carried out;

and it will be particularly important to have this information before largescale applications of the use of recombinant molecules is attempted.