Thurs 2.27.75

Singer

Provisional Statement of the Conference Proceedings

History: Introduction

1. This meeting was organized to review scientific progress in the area of recombinant DNA molecules and to discuss appropriate ways to deal with the potential biohazards of this work. We have heard about the enormous scientific progress already achieved in this field, and (We) have seen glimpses of the remarkable potential of these methods to accelerate the rate at which ~~we may gain~~ understanding (may be gained) of the fundamental processes occurring in eukaryotic 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.

The participants at the meeting agreed that the pause in research, called for in the July 1974 committee letter, ought not to be left unresolved. They considered whether there were ways in which the scientific work could be undertaken with minimal risks to the workers in laboratories and to society at large. It was emphasized that, in the longer term, even more difficult problems may arise in the probably large scale applications of this work in industry, medicine and agriculture. Even in the currently more limited area of the conduct of research in this field, the evaluation of potential biohazards has proved to be extremely difficult. The new techniques combining 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, the work should proceed but with appropriate safeguards. Although future experience may dispel many fears, standards of protection should be set high at the beginning and each escalation, however small, should be carefully assessed.

General Principles

2. 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 ~~of~~ from any risk. Reasonable principles for dealing with these potential risks are to adopt containment as a part of the experimental strategy and that the effectiveness of the containment should match the risk. Whatever scale of risks is ultimately devised, we shall need a commensurate scale of containment. Consequently we must seek means for estimating the risks, perhaps subjectively at first but objectively as we acquire additional knowledge, and then to match that risk (to) the appropriate degree of containment. (with) Experiments requiring large scale operations would seem to be riskier than the equivalent experiment done on a small scale, and, therefore, require more stringent containment procedures. Improvements in the methodology, e.g., a "disarmed" vector or host cell, could permit a reduction in the containment requirement. Quite possibly the ways in which potential biohazards and different levels of containment are matched may vary from country to country; also, the ways could vary from time to time as the containment technology is improved. Thus, it is essential that there be a continued reassessment of the balancing of risk against level of containment.

some standard

Multiple factors define each level in the scale of containment. The most important factors, because they contribute most significantly to limiting the spread of the recombined plasmids, are biological barriers. These barriers are of two types; fastidious bacterial hosts unable to survive in natural environments and non-transmissable vectors designed to grow only in specified hosts. A second factor is adherence to good microbiological practices which, to a large measure, can limit the escape of organisms from the experimental situation. Physical containment, exemplified by the use of suitable hoods, or, where applicable, contained laboratories is an additional factor. Further, education and training of all personnel involved in the experiments is essential to the effectiveness of all of the above.

Include decrease in scale as a factor

3. Specific Recommendations

These recommendations reflect the principle that a serious evaluation of biohazard potential and the adoption of appropriate biological and physical barriers are integral parts of experiments with recombinant DNA molecules. In this section three levels of containment affording increasing protection are defined. Then, various types of experiments are evaluated for potential biohazards and matched with appropriate containment levels.

A. Types of Containment

The types of containment are an adaptation of the "NCI Safety Standards".

1) Low-This type of containment involves basic good medical microbiological techniques. The essential factors are wearing lab coats, using mechanical pipettes, no eating in the lab, and that sonication and other procedures which generate large aerosols should be done in biological safety cabinets.

While existing vectors can be used for this level of containment, as safer vectors become available, their use is recommended.

2) Moderate-Both physical and biological containment enter into this containment system.

The physical containment for moderate risk agents was designed for handling moderate risk oncogenic viruses. The main features are that transfer operations are to be carried out in biological safety cabinets, gloves are worn in addition to lab coats, vacuum lines are protected by filters, and negative pressure is maintained in limited access laboratories.

The physical containment procedures are recognized to provide significant but incomplete protection against the accidental spread of biological agents. The potential hazard of moderate risk agents is such that a strong measure of biological containment is needed to ensure their safe handling. Therefore, experiments with such agents should only use prokaryotic vectors which have been designed with increased safety in mind. Such vectors are currently being designed and created, ~~and should be available in the near future so that these experiments can proceed~~.

3) High-This type of containment involves facilities which are isolated from other areas by air locks, clothing changes and shower rooms and which have treatment systems to inactivate or remove biological agents that may be contaminants in exhaust air, liquid and solid wastes. The handling of agents shall be confined to biological safety cabinets and all persons occupying these areas shall wear only laboratory protective clothing and shall shower at each exit from the containment facility. In addition the containment facility shall be maintained under negative air pressure.

Again, only vectors designed for safety should be used.

B. Types of Experiments

The letter published by the Committee on Recombinant DNA Molecules in July 1974 requested that the scientific community join the Committee in deferring two types of experiments. The letter also advised caution regarding a third type of experiment. In the following assessments of potential hazards the three original categories are maintained, but with some redefinition.

1) Prokaryotes - Potential biohazards of experiments involving genetic exchange among prokaryotic arganisms can in general be accurately assessed.

Experiments involving organisms that normally exchange genetic information involve no novel biotypes and pose no hazards that cannot be contained by the standard microbiological laboratory techniques appropriate for the handling of these organisms.

Experiments involving the introduction of bacterial genes into species in which they have not been found to occur naturally result in novel biotypes and so pose increased potential biohazards. Such experiments involving genetic determinants affecting pathogenicity for man or other species or antibiotic resistance should be undertaken only under conditions of moderate or high containment; any large scale industrial, commercial, agricultural or other applications should be deferred pending the issuance of appropriate official guidelines by national scientific bodies.

2) Viruses - Experiments employing low risk animal viruses as vectors to introduce new genetic material into animal cells can be carried out under moderate physical containment conditions.

Experiments involving linkage of viral genomes or genome segments to prokaryotic vectors and their introduction into prokaryotic cells should be carried out under moderate containment conditions ~~including improved vectors~~. Rigorously purified fragments of the demonstrably non-transforming regions of oncogenic viral DNAs or regions of non-oncogenic viruses could be attached to ~~plasmid~~ DNAs and introduced into E. coli under moderate risk conditions with existing vectors.

Experiments involving high risk viruses should be carried out under high containment.

Aaron: We see no justification for experiments with high risk viruses.

incorporate difference in risk categories depending on whether or not expressed, ie: lower risk of toxin involved.

3) Eukaryotic DNA - In the low risk category are most experiments involving the fusion of prokaryotic vectors with DNA from ~~prokaryotes~~, lower eukaryotes, plants, invertebrates and cold-blooded vertebrates. Moderate risk experiments involve the joining of DNA of warm-blooded vertebrates to prokaryotic vectors, ~~or the construction of recombinants between animal vectors and any DNA~~. Experiments with a high risk include the fusion of eukaryotic or prokaryotic genes to prokaryotic vectors when the resultant organism is likely to express ~~a toxic or a pharmacologically active agent.~~ an agent toxic to the ~~organism~~ host

toxic to who?

4. Implementation

It is clear that in many countries steps are already being taken by national bodies to formulate and establish guidelines. Until such guidelines are established we urge individual scientists to use the present document as a guide. There are in addition some recommendations which need not wait until that time and which the scientific community could implement directly.

A. Development of Vectors and Hosts.

One of the most important and interesting accomplishments of the meeting was the beginning of the design and construction of bacteria and vectors which could radically improve the safety of these procedures by many orders of magnitude. It is certain that in the near future there will become available special variants of λ phage, non-transmissible plasmids and special E. coli which will not only optimize safety but will also bring about considerable technical improvements in the methods themselves. Other bacterial systems, particularly suitably 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. We think that work which aims to improve the vectors or hosts should be given high priority.

Pledge of availability of organisms

B. Laboratory Procedures

Responsible investigator must see to this-

Before any experiments of this kind are initiated, the research staff of the laboratory shall be informed of all of the hazards that might be associated with such experiments. Laboratory workers must also be trained in the containment procedures that are designed to control the hazards. This training must include emergency procedures that are to be performed in the event of an accident. It is also strongly recommended that appropriate health surveillance of all personnel, including serological monitoring, be conducted periodically to establish a base for epidemiological analyses.

consent?

could be misleading - Young

C. Education

A continuing reassessment of these problems to take into account new and developing scientific knowledge is essential. This could be achieved by a series of annual courses, workshops and meetings which would also serve to train individuals in the relevant methods. Consideration should also be given to the establishment of a newsletter for the rapid dissemination of new information pertinent to the effectiveness of biological containment.

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5. Proposed Model Containment Review Process

A review process should be established which would be able to determine whether a given laboratory has the appropriate containment facilities for a given type of experiment. As far as possible, the biohazard review process should not lengthen the time required for review of research proposals. The specific form of the review procedure in different countries for different scientific and industrial laboratories must depend on local circumstances. The following proposal is, therefore, presented as a model. The model is designed for universities in the United States but would have to be modified for other situations.

Each university or research institution should have a committee empowered and trained to grade the physical containment facilities of its laboratories (e.g., low, moderate or high according to established guidelines). The local committee would provide the laboratory head with a statement certifying the containment rating of the laboratory(subject to periodic reevaluation).

When an individual applies to an agency for funds to support work on recombinant DNA molecules, the certificate of containment rating would be appended. The group reviewing the grant would then determine whether the certified level of containment matched whatever biohazard might result from the proposed work. The biological barriers incorporated in the experiment, the magnitude of proposed growth of bacteria, the type of DNA to be cloned, etc., would all enter into the decision. If the reviewing group is satisfied, the grant would be processed for scientific merit in the usual fashion. If a question arises concerning the appropriateness of the certified containment level, the NIH Advisory Committee on Recombinant DNA Molecules or some other body would be asked for an opinion or ruling.

This procedure would not guarantee that all experiments would be performed under the required containment conditions but if the investigators have reasonable good will, the system would generate widespread compliance.

6. New Knowledge

This document represents our ~~best~~ first assessment of the potential biohazards in the light of current knowledge. To improve this assessment, it will be important to have answers to the following questions.

(1) Are eukaryotic genes or viruses expressed in prokaryotic hosts and, if so, can they modify the biohazard potential of these cells?

(2) Can free DNA molecules infect animals or plants?

(3) Can prokaryote-eukaryote recombinant DNA molecules, either free or encapsulated as phage particles infect animal or plant cells and be expressed there?

(4) Can mammalian cells in culture be genetically transformed by free homologous or heterologous DNA?

(5) Can hybrid animal virus DNA or virus-plasmid hybrids cause tumors in animals?

(6) Can methods be developed to monitor effectively the escape and dissemination of cloning vehicles?