international news

Berg conference favours use of weak strains

from Colin Norman, Pacific Grove

FUTURE historians of science may well record that a highly significant event took place in a California state park between February 24 and 27, 1975. Meeting at the Asilomar Conference Center here for three days of intense—and at times heated—discussions, 140 scientists from 16 countries agreed that strict controls should be placed on an exciting new technique which enabled genes from one organism to be transplanted directly into another.

This attempt at self regulation by a group of scientists is believed to be without direct precedent, for although restrictions have been imposed on research before, they have usually resulted from concern over the uses to which science will be put, or from public pressure. In this case, however, the call for controls has come from the very scientists most actively engaged in the research and it has come at the inception of a new field of study, before any known hazards have arisen.

Although the genetic transfer technique promises revolutionary advances in molecular biology, the conference decided that its use should be tightly controlled because the genetically modified organisms it creates may pose direct, but unpredictable, health hazards. Such concerns led a group of American biologists, headed by Paul Berg of Stanford University, to issue a call last July for a world wide suspension of research involving the technique, until the possible hazards associated with it have been evaluated (*Nature*, **250**, 175; 1974). This conference was called to try to assess the hazards and to decide under what circumstances such research should be resumed.

The meeting concluded, in short, that some genetic manipulation experiments could go ahead immediately, provided fairly strict safety precautions are adopted, but that others should not be attempted until safer procedures have been developed. It was also agreed that there are some types of experiments which are potentially so hazardous that they should not be carried out under any circumstances. A statement of the principal conclusions reached at the meeting is now being drawn up.

It was remarkable that even a statement of general principles was approve by all but a tiny handful of participants at the conference, for at times it seemed inevitable that there would be a disastrous split between those who wanted to press ahead with their experiments and those who were arguing for restraint. In the end, however, the rift was healed by the realisation that safer procedures could be developed very rapidly to allow virtually all the planned experiments to proceed, so that any continuation of the suspension of experiments would be short lived. The nub of the matter is that gene transfer experiments could be carried out much more safely using biologically disabled microorganisms which would be unable to survive outside the laboratory, instead of the organisms that are commonly used in genetic research. Fortunately, it was generally agreed that by using what one participant described as "old fashioned steam genetic engineering", such enfeebled strains could be developed and produced in quantity in a matter of weeks, so that even the most gung-ho researchers were able to accept that it would be prudent to delay some of their riskier experiments until safer organisms are available.

Coming as they do from the people who will be most affected by them, the proposed controls on such experiments may seem like the classic case of the fox being set to guard the chickens—in fact, they have already been criticised as such by a group of radical scientists known as Science for the People. But the recommendations are much stricter than might be expected and, if implemented, they would require expensive modifications to many laboratories hoping to conduct gene transfer experiments. They are also more stringent than recommendations published last month by a committee of British scientists, chaired by Lord Ashby, which concluded that although development of safer strains of experimental organisms would be desirable, present safety precautions would probably be sufficient to allow most experiments to proceed.

The technique in question, according to Sydney Brenner of the MRC Laboratory for Molecular Biology, Cambridge, "is going to generate the most exciting period, I think, in biology, and it is going to last for at least 10 years, maybe more". It involves use of a newly discovered class of enzymes, called restriction enzymes, to snip fragments of genetic material (DNA) from one organism and splice them to the DNA of another organism, such as a virus or bacterium.

The key to the technique is that some restriction enzymes sever one strand of the double-stranded DNA leaving what are known as 'sticky ends'. Two DNA molecules cut by the same enzyme will have identical ends, which can be joined together and annealed into a single molecule. Three types of experiment using this method of cutting and splicing together DNA fragments from different sources have either been carried out or are being contemplated. The first involves insertion of 'foreign' DNA fragments into a package of DNA called a plasmid, which can replicate inside a bacterium independently of the bacterium's chromosomes. A second type of experiment involves splicing fragments of foreign DNA into the DNA of viruses called bacteriophages, which attack and replicate in bacteria. And a third possibility involves joining together pieces of DNA from two different viruses, to form a hybrid virus.

The scientific promise of the technique is that it will allow fragments of DNA which are believed to be responsible for initiating specific chemical reactions to be inserted into a bacterium and copied every time the bacterium divides. The bacterial culture would then produce large quantities of the foreign DNA fragment, which could be studied more easily. The technique also offers the possibility of taking particular genes out of their normal environment and studying how they operate in the simple genetic system of a bacterium. And the construction of hybrid viruses may help to shed light on why some viruses cause tumours in animals.

In addition to that potentially rich scientific harvest from such genetic manipulation experiments, the technique may offer the more remote possibility of isolating genes which code for nitrogen fixation in leguminous plants and introducing them into the cells of crops such as cereals. If that could be accomplished the resulting hybrid cereals would no longer need a constant supply of nitrogen fertiliser. Another widely touted possibility is

that it may be possible to mass-produce the genes responsible for promoting the synthesis of insulin by growing them in bacterial cultures.

But the technique also involves the possibility of significant health hazards. The essence of the matter is that by combining fragments of DNA from different sources an infectious organism might be produced endowed with unpredictable biological properties. By splicing genes from two viruses, for example, the host range of the virus might be extended, so that a virus which does not normally infect might inadvertently be made to do so. The consequences of transferring segments of DNA from animal tumour viruses to viruses which commonly infect man, for example, could be disastrous. The chief reason for concern with such experiments is that segments sliced from a molecule of DNA by a restriction enzyme contain considerable amounts of genetic material in addition to the particular gene which is being studied, and the effect of that unknown DNA cannot be predicted in advance.

Another cause for concern is that the bacterium most commonly used in genetic research is a laboratory strain of *E. coli*, a bacterium which is normally present in the human gut. Although Dr E. S. Anderson, of the Enteric Reference Laboratory, Colin Dale, presented evidence suggesting that the particular strain of *E. coli* used in laboratory research does not survive for long in the gut in competition with other strains, it was universally agreed that more reliable safety factors are needed.

That realisation led to perhaps the most productive session at the conference—a session during which specific mutations were discussed which could be built into laboratory viruses and bacteria used for genetic research, to ensure that the organisms would be incapable of surviving outside the medium in which they were cultured.

According to Waclaw Szybalski, of the University of Wisconsin, such biologically enfeebled strains could be produced "by the time we have slept off this meeting." In short, the mutations would involve making bacteriophage lambda (the most commonly used bacteriophage for genetic manipulation experiments) unable to survive at human body temperature, unable to grow the tails needed to make it infectious, and able to infect only the particular modified strain of bacteria used in the experiment. *E. coli* could also be equipped with genetic mutations which would make it unable to synthsise diaminopimelic acid (DAP), a constituent of cell walls, so that it would have to be provided with DAP in order to survive. *E. coli* could also be made temperature-sensitive.

With the promise that such disabled organisms could be produced and made widely available in a matter of weeks, much of the concern about delaying experiments, which had been voiced during the first two days of the meeting, was considerably diminished. Consequently, a draft statement, drawn up by the oragnising committee and discussed on the final day, was accepted by an overwhelming majority of the participants, even thought it called for many important experiments to be put off until safer laboratory strains of bacteriophage and *E. coli* are developed.

The draft statement outlined three levels of safety precautions which should be accompanying various genetic manipulation experiments. Only the lowest risk experiments should be carried out with existing strains of bacteriophage lambda and *E. coli*, the statement suggested, but they would require safeguards such as autoclaving all cultures, safe pipetting procedures and so on. Such experiments would consist of gene transfers between organisms which normally exchange genetic material—in other words, no novel genetic combinations would result from the experiment—and the insertion of genes from invertebrates and cold-blooded vertebrates into bacteria.

The next level of experiment—those embodying moderate risk—would require use of biological safety cabinets, negative pressure in limited access laboratories and use of some protective clothing. Estimates of how much it would cost to equip a laboratory to conduct such experiments varied widely, but it was generally reckoned that at least $20,000–40,000 would be needed. In addition because physical safeguards cannot guarantee absolute protection against the spread of biological agents, such experiments should await the development of enfeebled strains of viruses and bacteria, the statement suggested. Experiments such as the splicing together of DNA from viruses, linkage of viral DNA to bacterial plasmids, and the joining of fragments of DNA from warm-blooded animals to bacterial DNA would fall into this category.

The highest risk experiments which could be done would again require the use of enfeebled strains of bacteria, and they should only be conducted in special laboratories equipped with airlocks to isolate them from other areas. Protective clothing should be worn, and showers would be required on entering and leaving the laboratory. Examples of such experiments are the fusion of genes to bacterial DNA when the resulting organism is likely to produce an agent toxic to the host, and work on viruses such as smallpox, which present a high risk in themselves.

Finally, after much debate, it was agreed that there are some types of experiments which should be ruled out altogether. Although such experiments were not defined, they would include the insertion into *E. coli* of the gene which specifies production of botulinus toxin. There would, however, be little justification for carrying out such experiments, except to produce lethal agents for biological warfare.

A statement setting out those general principles was agreed to by all but about three or four participants. Among the dissenters were two Nobel prize-winners, Joshua Lederberg, of Stanford University, and James Watson, of Cold Spring Harbor, both of whom had consistently challenged some of the basic assumptions underlying the conference.

Lederberg, for example, said in a statement he distributed at the conference, that "research on recombinant DNA is, in my opinion, the central way in which molecular genetics can contribute to the solution of important medical problems" and he warned repeatedly that any delays in carrying out the research should take into account the fact that important benefits may also be delayed. He also suggested that regulations governing such research are likely to be frozen in place, so that it would be difficult to make them less stringent later if it turns out that the risks have been overestimated. Lederberg also took exception to the classification of experiments into only three classes, pointing out that most of the contemplated studies would fall into the low or moderate risk categories, but the difference between the two is "considerable reconstruction of a laboratory".

Watson drew attention to the discrepancy between regulations being proposed for genetic manipulation studies and those governing work on tumour viruses. "As someone in charge of a tumour laboratory", he said, "we are working with something I feel is instinctively more dangerous than anything I have heard here", yet the regulations governing research on tumour viruses are relatively lax. "I think you should just use common sense", he said, but added that "you will have to live with the fact that somebody may sue you for $1 million if you are careless".

Underlying much of the discussion of the need for self-imposed controls was acceptance of the fact that science has lost much of the public support that it once enjoyed, and that if regulations were not imposed from within, legislation could be anticipated which would probably turn out to be much more restrictive. Whether this unprecedented attempt at self regulation will work, however, remains to be seen. □