p224
ORIG.

[Ms. of article submitted to PRISM --AMA policy journal]
JUNE 15 1975

DNA Research: Uncertain Peril and Certain Promise

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Nucleic acids were first isolated from cells and recognized as distinctive substances over 100 years ago . However, their crucial role in biology was long obscured for two reasons:

1) a methodological one -- the lack of a direct biological assay for the specificity of a nucleic acid; and

2) a doctrinal one -- the fallacy of chemical simplicity that characterized the organic chemists' model of nucleic acids as homogeneous polymers devoid of informational variety.

As a result, although nucleic acids were known quite early to be systematically associated with the chromosomes (indeed these are named from the colorability of nucleic acids with the basic dyes used in cytology) there seemed to be no way to relate them to the vital functions of the cell, much less to put them to any utilitarian application in medicine, in agriculture, or in industry.

The scientific revolution of modern biology may be dated to 1944 when O.T.Avery, Colin MacLeod and Maclyn McCarty working at the Rockefeller Institute announced that the "transforming agent" of the pneumococcus consisted of DNA. This agent had already been speculatively compared with genes transferable from one bacterial strain to another. The announcement quickly provoked a high level of critical discussion, as befit a claim of such fundamental import. It was crucial to be certain that DNA itself,rather than a trace contamination with an active protein, was the active agent. Furthermore, too little was known of bacterial genetics to be sure that an isolated example of the modification of a bacterial outer coat really did reflect a gene transfer. However, so many new experiments were prompted by these new claims that, by 1953, the detailed atomic structure of DNA had been embodied in the now familiar double-helix model of Watson and Crick; and a whole new discipline of microbial (and viral) genetics had grown up in an area that a few years before had been widely thought to be wholly disconnected from Mendelian-Darwinian biology.

The unprecedented acceleration of fundamental chemical biology since 1945 can be related to the intellectual stimulation of these findings, and the analytical tools that accompany them, as well as to the policy of continued federal support of scientific research in the era since World War II. We may also note that medical microbiology was just then past its most heroic era, the identification of all of the principle agents of infectious disease, the very empirical success of which may have tended to hinder closer cooperation with academic biology.

Although our scientific understanding of the cell has been completely transformed in the last 30 years, we have yet to see practical applications of molecular biology of nearly comparable importance. There is very little indeed in the practice of contemporary medicine (even of clinical genetics) that depends on the knowledge that

DNA has a bihelical structure, which is an appropriate shibboleth for the underlying doctrine of this new field. This has not eroded our faith that basic approaches to the understanding of viruses, of the neoplastic and of the aging cell, of the mechanism of the immune response, or of aberrant chromosomes, will be instrumental to farreaching changes in medical technology. The human benefit from these will match the theoretical impact that DNA study has already demonstrated for cell biology.

These expectations for a possibly long-delayed future fruit have been exalted within the last year or so by some new findings that give us much greater technical capacity for the laboratory manipulation of microbial DNA. These new methods of "DNA-splicing" have already opened up many lines of experimental investigation of the structure of eukaryotic [higher life forms] chromosomes. For it is now possible to fragment an animal or human cell's DNA into perhaps a million segments, and transfer a single one to a bacterial host wherein it can be studied in a microcosm, or whence large quantities of a specific DNA can be produced for more elaborate analysis than will ever be possible with the enormously complex original source material.

This technique of gene implantation can also be used to transfer the genetic information for a given product from one species of cell to another; and this is the direction that, in my own view, leads to an early chance for
a technology of untold importance for diagnostic and therapeutic medicine: the ready production of an unlimited variety of human proteins.

Analogous applications may be foreseen in fermentation processes for cheaply manufacturing essential nutrients, and in the improvement of microbes for the production of antibiotics and of special industrial chemicals. It has also been foreseen by many workers in the field that such technical potency may also be associated with public hazards, and that we then face a Promethean dilemma.

Public policy decision in this field can only lead to social benefit if it is well informed,equally about the potential risks as well as benefits of further work on DNA-splicing. Indeed, if any substantial risk can be identified, there is no question of the need for ethical and operational standards; the only question must be whether their actual form and implementation result in a true net advantage to the public health and safety. Too often, the superficially easy way to cope with such a problem is to invoke a formal regulatory statute, ignoring how well the actual bureaucratic implementation or policing of the rules meets the intended balance of risks and benefits. Before elaborating on the policy issues, it may therefore be incumbent to outline some further detail on 1) the present state of DNA-splicing technology, 2) some promising applications, and 3) the sources of risk in further work in this field.

1. How we splice DNA in the laboratory.

DNA recombination as the be-all and end-all of sexual reproduction is of course one of the major happenings of the natural world of life.

Among higher forms, exchange of DNA is almost always limited to members of the same or closely related species and is generally associated with sexual differentiation, that is males and females. Bacteria and viruses exhibit some analogous barriers; but also many exceptions: which may reflect the fragility of the idea of "species" when applied to these forms. For example, the entire group of "enteric bacteria" including also such forms as Shigella, Escherichia coli, Proteus, and Serratia can readily be shown to exchange genetic fragments without special interventions -- we have to assume that this also occurs in nature,

and that it would be difficult to aggravate the risks already latent in that natural occurrence.

An especially interesting and important level of genetic organization in bacteria is the plasmid: a bit of circular DNA that behaves like an extra small chromosome, and one that seems to survive in nature by virtue of its easy transmissibility from one bacterial strain to another. Many different kinds of plasmids are known; medically, the most prominent today are those which confer transmissible antibiotic resistance on human pathogens, notably staphylococci and some enteric pathogens like Shigella. These plasmids are part of the evolutionary history of their host organisms: the spread of antibiotic-resistance plasmids is the most formidable ecological response that pathogenic bacteria have yet discovered to our widespread use of antibiotics. Other plasmids are undoubtedly involved in altering the pathogenicity and host-specificity of various bacteria;therefore in simple self-defense of our species it is urgently important that we learn what we can about them.

Plasmids have now achieved special prominence also for a technical reason, that they are especially convenient vehicles for DNA splicing and for the transmission of DNA segments from one species to another, especially in conjunction with another elegant tool: the R-[for restriction] enzyme. The R-nucleases are widely distributed among cell types; they may be an important mechanism by which a cell fends off any "foreign" DNA while protecting its own. For historical reasons, this particular mode of defense has been called "restriction", and now that we know the responsible enzymes, they have inherited a name that may be confusing to bystanders. The important attribute of an R- nuclease is that it recognizes a special sequence of bases in double-stranded DNA, depending on the particular enzyme, and that it will then cut that DNA leaving a protruded "sticky" end. The "stickiness" arises from the fact that the same sequence can be recognized on other protrusions, and that the Watson-Crick rules for uniting separated DNA strands will then apply.

For example, Dr. Stanley N. Cohen has used an R-enzyme to simplify a naturally occurring plasmid to the point where it consisted of a small circle of DNA, embracing the minimum genetic information needed to be able to replicate, plus a single R- enzyme recognition site. This artificial plasmid, pSC-101, has been an important tool for DNA-splicing research. When it is exposed to R-enzyme, the circle is cut into a single open length with sticky ends. Another enzyme, ligase, can be used to rejoin such ends; However, it is also possible to insert other sticky- ended pieces of DNA from divers sources into the plasmid, then close it up with ligase. This is then the key to the convenient design and construction of new DNA molecules which can then be transferred to a bacterial host -- an experiment which is today's counterpart of Avery's report of 30 years ago.

The new DNA does not have to be of the same bacterial species; for example, Dr. Cohen and his collaborators have already reported on the effective transfer of DNA from a toad, Xenopus, into E. coli with evidence of the production of toad- like ribosomal nucleic acids by the modified bacteria.

Besides these plasmids, bacterial viruses are being used in a similar fashion; less elegantly, segments of DNA from intact bacteria may also be used both for insertions and as the acceptors. All of these experiments so far depend on the innate (and poorly understood) ability of bacterial cells to incorporate DNA furnished from without. There have been many published claims of similar phenomena with plant and animal cell acceptors, but none to date have the reproducibility and general acceptance as undoubted fact that pertains to the bacterial work. There is no immediate substance to the idea that these

techniques are applicable to the "genetic engineering of human beings". (In the long run, the possibility of such technical capabilities cannot be denied in principle, no more than we can disprove the possibility of a peaceful world, or of a global morale capable of the wisest disposition of our existing powers for good and evil.)

The special power of these enzymological techniques is that they depend on the basic chemical structure of DNA (recall the shibboleth!) rather than on the biological adaptations of particular microbial species. Laboratory manipulations may then synthesize constructs that occur rarely, if ever, in the natural world. It is difficult, however, to assess just what can or cannot occur in the entire immense mass of the earth's biosphere. R-enzymes,mixed DNA and acceptor bacteria surely occur with some frequency in natural habitats. The likelihood of a prevalent natural transmission of plasmids among "unrelated" forms is also enhanced by recent findings of agents with extraordinarily broad host range. From a practical standpoint, there can be no doubting the power of DNA-splicing as a means of acquiring specific gene sequences and studying and applying them. Possible regulatory strictures against promoting the transfer of plasmids among "species that do not normally do so" may be mooted by the behavior of the newly found agents -- unless, of course, we are prevented from making just such discoveries by the restriction of research.

DNA-splicing is however but the most powerful of a range of techniques and processes whose end-effect is the bringing together of more or less natural assemblages of DNA information. Indeed, it may prove to be less powerful than older methods -- of sexual crossing, of transduction with bacteriophage, of DNA transformation -- for special constructions that involve larger complexes than the segments yielded by the R-enzymes. These methods in turn are an extrapolation of the artificial breeding of domestic animals and plants upon which civilization was founded. In any event, the most efficient application of DNA- splicing requires intimate knowledge of the genetic structure of both the donor and the acceptor strains, for which breeding methods are important if not indispensable.

Perhaps the most important single conclusion is that this technology is just in its infancy, but has already made great leaps; and that it is simple enough that it can be practised in any laboratory that can handle pure bacterial cultures. Just this simplicity, which makes for great convenience and rapidity of experimental advance, has been a source of concern about the proliferation of the methods in the hands of people with less than mature professional and ethical judgment and with deficiencies in the skills entailed in containing bacterial cultures in the laboratory.

2. The promise: biosynthetic human proteins

DNA segmentation and splicing is certain to play a vital role in the further domestication of microbes for such roles as antibiotic development and production of high quality protein supplements. However, the unique strength of this procedure is to allow the large scale production of gene products of a less easily domesticated species: the human. In particular, human proteins already play a substantial role in medicine but one which is hindered by scarce supplies.

The most attractive options that are visible are the human antibody globulins. This preference has a theoretical basis. Of all the body proteins, the immune globulins

have evolved to be variable within and between individuals in the fulfillment of a vital function. Compared to the rare genetic defects in other proteins (like hemophilia), failures or errors in production of antibody globulin are quite prevalent, and are known to play a major role in (1) defense against infectious diseases, (2) autoimmune and allergic disease, and (3) perhaps also in cancer.

The most comprehensive role of biosynthetic proteins would be in passive immunization against infectious diseases. Animal antisera were once used but had to be abandoned because of the anti-animal antibody that they provoked in man. Priority targets for passive globulin therapy are those diseases where either technical or social factors may lead to gaps in protection by active immunization. They include influenza, hepatitis, smallpox, encephalitis virus, rubella, herpes, rabies and perhaps also trypanosomes, malaria, schistosomes, tuberculosis and leprosy and many others.

I believe there is reason for special urgency to develop a backup capability of passive immunization to prevent a global catastrophe that may result from our becoming too complacent about active immunzation against diseases like smallpox and polio, and the technical inadequacy of vaccines like rubella and hepatitis. Our general posture of defense against viral pandemic is a feeble one. We have no assurance that the next influenza epidemic will not be slightly more virulent and cost a million lives for lack of a ready defense.

A broader need applies to polyvalent prophylaxis for infants. The principal medical argument for breast-feeding is the provision of colostrum and of a continuing supply of maternal mixed globulins in the milk. There would be a huge and valid market for polyvalent gamma globulin supplements to infant dietaries both in industrialized and in poorer countries. An analogous veterinary demand speaks to further efficiency in food production.

Specific antibodies are, of course, already very widely applicable as diagnostic reagents of high specificity and selectivity. Blocking antibodies may also be expected to play a useful role in protecting transplanted tissues and organs from more aggressive immunological attack by the new host. Conversely, tissue-specific ligating antibodies, although not necessarily themselves carrying cytotoxic capability, may also be expected to be useful in enhancing the cell specific toxicity of cancer controlling compounds. Cell specific reagents will also be invaluable for diagnostic purposes and for the specific separation of human cell types to be used further for either diagnostic or therapeutic applications.

Anti-sperm immunity is also being very seriously proposed as an approach to durable male contraception. I have been quite uneasy about such proposals that involve the vaccination of men against their own sperm for fear of unwonted side-effects and also on account of probable difficulties in reversibility. Passive antibody directed against sperm flagella is demon- strably able to interfere with fertilization simply by the immobilization of the sperm and should have a minimum of other side-effects. Such immunizations would be reversible by the spontaneous decay of passive immunity over periods of from 3 to 6 months. Comparable possibilities exist for the immunization of

women against sperm.

Besides the specific antibody globulins, a number of important but less specific proteins (complement, properidin) play an important part in defense against infection. Fibrinolysin (plasmin) and urokinase (plasminogen-activator) represent a group of enzymes that are experimentaly promising for the control of embolism. Many protein hormones are equally scarce for clinical trials. The list could be extended substantially! The most important products are perhaps those that are invisible by present methodology.

Microbial biosynthesis may well be supplemented by organic synthesis by the elaboration of proteins in human and hybrid somatic cell culture and by cell free ribosomal synthesis with contrived m-RNA. Each of these options has its own peculiar difficulties and hazards and the whole field will be most rapidly advanced by using the best available methods for a given problem. -------

3. The risks: will dangerous organisms escape?

At the present time, perhaps a half-dozen bacterial species are well enough understood to be prime vehicles for laboratory study of DNA-splicing. For safety and convenience, investigators have preferred not to use pathogenic forms wherever feasible. Significant concern arises from the possibility that the introduction of new genetic information may (inadvertently) generate a new pathogen for man, or its analogue, a source of ecological disruption at some other point in the biosphere. The most likely, but not necessarily the only, source of such genes for pathogenicity are precisely the organisms that most urgently need further study -- the subtle and insidious killers that are not now amenable to medical treatment and prevention. These include slow virus infections that may be involved in a wide range of chronic diseases and cancer, and more familiar viruses like herpes for which satisfactory vaccines are not now available.

Public discussion of advances in DNA-splicing indeed have been almost totally focussed on the possible hazards of escape of new forms of microorganisms, rather than on their utilitarian merits. The most urgent source of concern has been for the prospect of introducing potential cancer-causing DNA into common bacteria. While it is recognized how speculative this hazard is, the general territory is so poorly understood that no one can argue against the need for cautious laboratory procedures. Having gone so far, a number of workers -- particularly those not previously experienced or trained in medical microbiology -- have confessed having given almost no thought in the past to problems of microbial safety; and some of these are now among the most zealous in demanding tight regulation of further research. As part of this process, there has been a sincere ,almost frantic, effort to seek out the most remote conceivable hazards.

Viewed as a rather public soul-searching and self- education, these discussions are invaluable. The main danger is that tentative questions will be incorporated by some political imperative into iron-clad regulations that will be with us long after anyone has forgotten why they were instituted. One can after all raise similar questions about the widest range of human activities: should it be lawful to keep domestic cats now that they are under suspicion of harboring toxoplasmosis, and possibly leukemia as well? The same kinds of questions that are asked of microbiology could be lodged against plant breeding: what positive assurance can there be against the next artificial pollination

producing the weed that will ruin the wheat crop a decade from now? Closer to home, should we forbid international travel, given the certain knowledge that our quarantine procedures are quite unable to hinder the importation of exotic diseases?

For each of these cases, and many more, the apparently innocuous doctrine: "As long as there is any risk, don't do it!" can only lead to a loss in human welfare. We must instead make every feasible effort to assess both the risks and the benefits of a given course of action -- only then are we in a position to weigh the optimal balance. This in no way may deny the rights of individuals to make voluntary decisions about their exposure to risk, even if for public benefit. But individuals can hardly make the best policy about their own future, including their expectations for what medicine will offer for the infirmities of their own later years, without expert assessment.

These assessments are difficult, problematical, and controversial but a committee of the National Academy of Sciences has made some headway in trying to classify different categories of hazard. Where that hazard is reasonably predictable (in the current atmosphere, now so highly sensitized) laboratory containment precautions akin to those appropriate for known pathogens have been recommended, and these will doubtless be comprehendingly complied with. This applies, for example, to experiments involving the recombination of known tumor virus DNA with bacterial plasmids.

For more conjectural hazards -- like the introduction of antibiotic resistance into common, non-pathogenic species -- the requirements for high security laboratories may be an inordinate burden (who, in fact will pay for them?) in relation to the prospective gains. The best strategy here seems to be the development of safe vectors: plasmids and bacteria engineered to have little chance of survival outside the laboratory. In fact, in the long run this is a safer procedure than relying upon uncertain human compliance with fixed rules and regulations.

Remaining controversies in this field center upon rather complicated analyses of the remotest kinds of risks. Given some additional time, and a decision to postpone a rigorous framework of external regulation, most research institutions will be able to work out their own reasonable plans based on the national guidelines. A premature crystallization of such regulation will not only frustrate the achievement of pragmatically useful goals,but will hinder the research needed to make more refined assessments. Those who regard themselves as guardians of the public safety must count not only the speculative hazards of these marginal situations, but also the costs to the public health of impeding their investigation.

This partly voluntaristic approach will not satisfy a demand for absolute assurance that no foolish experiment is ever attempted. But the history of human institutions should suffice to show that NO system of sanctions can have such a perfect outcome. The human species is constantly and inevitably attended by contaminating and parasitic microbes -- the person suffering from an enteric infection who fails to wash his hands, or the influenza victim who insists on going to work is behaving unethically, and to the peril of his fellows. But we would scarcely invoke serious regulatory sanctions in preference to public education except where there is an unusual public risk, and some evidence that an enforced quarantine was likely to yield a positive gain.

While a newly awakened scientific conscience is the main source of public discussion of the hazards of microbiological research, some of it also appears to be directed to the frustration of its benefits. For some such critics, any measure that might prolong lives (especially of our own citizens) is merely worsening the population

problem and the excessive consumption of limited world resources. Others are sure that any technological advance in a free-enterprise society will inevitably benefit only the already powerful and wealthy. Still others harbor deep-seated resentments against the professional and economic dominance of the medical profession. These motifs do not alter by one whit the actual substance of concerns about the hazards of research; we must understand their prevalence to foresee the ways in which the issue may be exploited for reasons quite far from scientific conscience.

Senator Kennedy has remarked that society must give its informed consent to technological innovation. The power of the purse is enough to enforce that doctrine, nor can there be any quarrel with it on ethical grounds. The relevant information surely includes the hazards of saying no to prospects of significant medical advances. The particular field of DNA- splicing research, far from being an idle scientific toy, or the basis of expensive and specialized aid to a few lives, promises some of the most pervasive benefits for the public health since the discovery and promulgation of the antibiotics.