SCIENCE POLICY IMPLICATIONS OF DNA RECOMBINANT MOLECULE RESEARCH

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WEDNESDAY, APRIL 27, 1977

House of Representatives,
Committee on Science and Technology,
Subcommittee on Science, Research and Technology,
Washington, D.C.

The subcommittee met, pursuant to notice, at 10:14 a.m., in room 2318, Rayburn House Office Building, Hon. Ray Thornton (chairman of the subcommittee), presiding.

Mr. Thornton. Good morning. Today we resume consideration of science policy implications of DNA recombinant molecule research. We began hearings on this subject March 29, 30, and 31 and during those hearings received testimony from a number of distinguished scientists on the basic biology of this research, on the potential risks and benefits of this research and on actions being taken so far by the Federal Government and the governments of other nations to regulate the research. These hearings provide us with a good bit of background information which we felt we needed before considering the broader science policy questions that are of major concern to this committee.

Today we are going to explore further some of the concepts touched upon in our earlier hearings, particularly those scientific facts from evolution and epidemiology which are relevant to the DNA recombinant molecule issue.

The subcommittee believes that these aspects of the issue deserve fuller public discussion. Some people have suggested that DNA recombinant molecule research is tampering with evolution or that it is creating new DNA sequences which have never before occurred in nature.

Two of our witnesses this morning are engaged in basic biological research which is central to these issues and it is at the forefront of research in this field. We would especially like those witnesses to address the potential for natural recombinant DNA and the concept of evolution at the molecular level.

Some people have also suggested that risks of new, unknown, and unpredictable diseases are too great to permit DNA recombinant molecule research to continue except under the strictest containment measures or perhaps even not to continue at all.

We have two witnesses knowledgeable in the field of epidemiology, the science which deals with the incidence, distribution, and control of disease. We have asked them to address this argument by presenting to us those facts which might be related to the potential spread of some
infectious DNA recombinant molecule should a laboratory accident occur.

Our first witness this morning is Dr. George Piezzenik from Rutgers University. I am going to call upon Mr. Hollenbeck, the ranking minority member of this subcommittee, and Representative from New Jersey, for the purpose of making the introduction.

Mr. Hollenbeck. Thank you, Mr. Chairman. Today and in the past there has been concern in the public about this issue. As we begin our second phase of hearings today on the science policy implications of this area of genetic engineering, our study is going to be expanded to include, I hope, relevant testimony concerning evolution and epidemiology, subjects which have not been widely discussed before us until now.

In this regard, I am very pleased to have the opportunity to introduce our first witness, Dr. George Piezzenik. He is a scientist at Rutgers University, the State University of New Jersey. He has recently jogged the scientific community with a researched and published theory which questioned and challenged the entire basis for the current concern over recombinant DNA research and genetic engineering.

He has worked at the Cambridge Laboratory of Molecular Biology for the past 6 years with such scientists as Francis Crick and Sidney Brenner. Recently his position has been presented in the Journal of the Origin of Life, and he has received recognition for that in several science publications and other publications such as Time magazine.

[The article referred to follows:]
I am grateful to him that he came down here to address us and to the university for permitting him to do so. I would like to welcome him here today.

Mr. Thornton. One of the colleagues of Dr. Pieznek, Dr. Sidney Brenner, has recently been honored and has been selected as one of the 15 foreign scientists who are designated as foreign associates of the National Academy of Sciences.

Please proceed.

[A Biographical sketch of Dr. Pieznek follows]

Dr. George Pieznek

Born: December 19, 1914 in Havana, Cuba to Dr. and Mrs. Emil David Pieznek.

Education: Phillips Academy Andover 1931; Harvard University A.B. 1935; University of Miami B.A. 1937; New York University Ph.D. 1942; Rockefeller University 1942-47; M.R.C. Lab. of Molecular Biology, Cambridge University—1947-1950, 1955 to present; Assistant Professor, Department of Biochemistry, Rutgers University. The State University of New Jersey, New Brunswick, N.J. 08903.

Area of Interest: Genomic selection and nucleotide sequence analysis.

STATEMENT OF DR. GEORGE PIEZNEK, NELSON HISTORICAL LABORATORY, RUTGERS STATE UNIVERSITY, NEW JERSEY

Dr. Pieznek, Chairman Thornton, Representatives Hollembek and Dunn, members of the Subcommittee on Science, Research, and Technology, I would like to thank you for inviting me to appear as a witness before this committee.

I have been with my brother, Steve Pieznek, Deputy Assistant Secretary of State for Management.

Mr. Thornton. We are pleased to have you at the hearing.

Dr. Pieznek. It is my understanding that the basic biology of DNA recombination molecule research has already been reviewed and therefore I shall address myself to the scientific question of molecular evolution.

I will try to demonstrate that DNA recombination molecule research is a form of artificial nucleotide selection and therefore a question of relative molecular evolution.

On July 1, 1868, Darwin and Wallace presented a joint paper at the Linnaean Society describing their concept for the evolution of species. Wallace derived his concept from previous investigations, Darwin arrived at the concept of evolution of species by observing patterns of similarity between species and variations within species.

The similarity between species led the concept to the concept of descent from a common ancestor. We have taxonomic similarity to other life forms, because we all come from a common ancestor.

The variation within species led to the concept of competition for resources and the survival of the fittest. These variants having competed and survived leave more progeny. These progeny carry the particular genetic characteristics that allowed the ancestor to survive in a particular environment or situation. This process Darwin called natural selection as opposed to artificial selection.

At this point we can ask ourselves: if DNA recombinant research is a form of artificial human selection of nucleotide sequences, is there a natural equivalent process?

Darwin contrasted artificial selection with natural selection in his "Origin of Species." He states:

Man can act only on external and visible characteristics; nature if will be allowed to purify the natural preservation or survival of the fittest cares nothing for appearances, except insofar as they are useful to it or being.

This history of science shows that Darwin did not know nor understand the genetic constraints placed on the degree of inherited variability. Gregor Mendel's work on the independent assortment of genes passed through Darwin's hands unread.

Therefore, Darwin's theory is the simplest concept that explains the observed similarity between species and the variation within species. The explanation he offers rests on the belief that the observed characteristics are inheritable and the number of progeny an organism leaves behind reflects its ability to survive as well as to make in a particular natural environment.

Though Darwin clearly states in the quote given above that nature cares nothing for appearances, it actually the competition he describes is phenotypic. The phenotype is that part of the organism that can be acted upon by the environment. In most cases it is the whole organism. The definition of phenotype as an expression of genotype was developed by the neo-Darwinians. The discovery of mutation and its later localization in DNA allowed an explanation of inheritable variation.

It is at this point we can ask ourselves the question, "What are the phenotypic characteristics of nucleotide sequences or what are the phenotypic characteristics of the genotype?"

The neo-Darwinian concept of evolution is as follows: a random mutation occurs in DNA. It is transcribed into mRNA, it is then translated into a variant protein. This protein affects metabolic or structural components in such a way as to create some change in the whole organism.

Whether that protein organism's genes are altered depends on its competitive advantage to the other organisms in the environment.

The environmental conditions in which the competitive or mating takes place determine whether that organism's genetic contribution survives. If one samples that progeny population and finds that the variant organism's traits have become a significant proportion of the new population, then a neo-Darwinian would insist that the variant characteristic has conferred a selective advantage even if he doesn't know what that advantage is.

Non-Darwinians have challenged the neo-Darwinian interpretation by saying that the fixation of a gene in a population is a consequence of small population size and drift. That is if an individual is a mutant in a population of 100 individuals then the frequency of the gene he carries is 1 percent of the population. If he and two other individuals move to another island then the frequency of that gene is now 2 percent of 50 percent.

Evolutionary selection has occurred to increase the gene frequency simply by reducing the effective breeding population size. These mutations that are not selected, or neutral mutations, according to Kimura, are those which have either synonymous codon assignments and or are similar amino acid replacements in proteins.

This non-Darwinian theory quantitatively explains the constancy of mutation rate and the high degree of protein polymorphism.
We can now ask ourselves the same question about artificially inserted DNA sequences: "Does the insertion of a foreign piece of DNA confer an advantage or is it neutral to the ability of DNA to replicate?"

The data Darwin was working with was basically descriptive gross morphology of organisms; the data Kimura worked with was protein sequences, which he considered a phenotypic measure. Both, however, would predict, but for exactly opposite reasons, that if one could examine nucleotide sequences directly they would be random.

The Darwinian rationale is that the variant is a historical accident that worked, as well as the observation that there are so many steps between the mutation which is considered to be random and its expression as an adaptive phenotypic that essentially the complexity makes the relationship between nucleotide order and phenotypic adaptiveness intrinsically nondeterministic and random.

The non-Darwinians believe that every mutation occurs randomly and is expressed, if neutral, and survives in a population as a consequence of random drift. Almost all molecular biologists, geneticists, and biochemists fall into a spectrum between these two views, depending on whether they think about this question in the first place. Evolution is the basic dogma of life. DNA sequences must find their proper place in evolution.

If Darwin had lived in this generation, he would have had access not to finches on the Galápagos Archipelago but to DNA nucleotide sequences. That is, if Darwin had direct access to genotype instead of to phenotype would he have seen them as random? Or would he have seen patterns of similarity between species as well as variability of sequences within species?

My contribution to molecular evolution was to study both the Darwinian and non-Darwinian approach and to disregard their certainty in the essential randomization of nucleotide sequences and approached nucleotide sequences as Darwin might have.

I was able to find evidence for various types of patterns at the nucleotide sequence level. These patterns I called constraints or restraints depending on whether the pattern was a consequence of syntactical function or structural function. Examples of such patterns are the simple symmetry pattern of palindrome-AUUUAAGUG-AAAUAUGAAUUA-the internal terminator constraint, and the recently published constraint on messenger RNA sequence as a consequence of a postulated tRNA interaction on which makes the genetic code a partially overlapping triplet code.

The last constraint implies that though the genetic code is universal, messenger RNA translation is species specific.

In order to explain the existence of these patterns at the nucleotide level it was necessary to postulate the existence of a specific type of selection which acts at the nucleotide level. Whereas natural selection is predominantly phenotypic, the existence of order at the nucleotide level suggests that there is a natural selection that is genotypic. This I call genotypic selection.

In genotypic selection it is the DNA molecules themselves which compete for their substrates, their ability to be replicated, transcribed and translated. This type of selection occurs in the intracellular milieu.

Genotypic selection imposes structural as well as syntactical constraints on nucleotide sequences. That each sequence is derived from a previously selected sequence also imposes a historical constraint on propery sequences.

Genotypic selection is to artificial DNA sequence selection, as recombinant DNA work is to artificial breeding. Therefore a DNA sequence which has survived in a milieu of let us say mammalian DNA polymerases, mammalian RNA polymerases, mammalian tRNA, etc., et cetera, will have a hard time adapting to an E. coli environment with E. coli RNA polymerase, E. coli RNA polymerase, E. coli tRNA, and so forth. The machinery of expression imposes constraints on which is to be expressed.

For example, if a Congressman wishes to introduce a bill which is of great benefit to the public at large he must first demonstrate to each committee, Congressman, and ades how that bill is of direct benefit to them individually or their constituents before that bill has a chance to become law.

So, too, with DNA sequences. DNA sequences must first have all the proper structural and syntactical characteristics for replication, transcription, and translation before the protein products are made. DNA polymerases will replicate certain sequences better than others; only those sequences have a chance of being transcribed. RNA polymerase will recognize certain sequences more efficiently than others, only these sequences will be transcribed; and, ribosomes will bind certain sequences and not others, only those that are bound have a chance to be translated. Transfer RNA will interact with codons in the context of a cell.

My perspective of the chance of an extreme taxonomic class of DNA expressing its information, is the equivalent of a bill passed in the Korem Congress becoming U.S. law. It would require careful planning and extreme manipulation and if passed, irrelevant.

Mr. Thornton. I think your example is a good one.

Dr. Piesczek. It is a double-edge example.

Therefore, given the perspective of genotypic selection the hazards, as well as the benefits seem less dramatic. However, there is the observation that DNA is a historical molecule and may contain vestigial information that goes back 4 billion years. It is the expression of vestigial sequences that may now become a reality.

The consequences of vestigial or even random expression of small polypeptides is unknown and yet highly likely at the present stage of technological competence.

At present, I do not see the clean and present hazard or benefit from artificial DNA selection. DNA recombinant work will be a small part of significant nucleic acid research. Most of the significant work will revolve around studying naturally occurring nucleotide sequences.

I believe the only contribution of this range of experiments will demonstrate that messenger RNA and tRNA coevolved.

At present, I would prefer to see a clean and safe policy in regard to the regulation of recombinant DNA work. That is:

One: Those involved in regulating, as well as advising, which experiments are to be sanctioned should not be scientists with a financial, whether direct or indirect, interest in the area.

Two: That the regulating board consist of informed lay public, journalists, political representatives, union representatives, and scientists not involved in nucleic acid work, genetics, or molecular biology.
Three: The background of members of the scientific advisory board be investigated to make sure that they do not have a history of morally repulsive experimentation. Nor should they, like Caesar's wife, give the appearance of wrongdoing.

Four: In order to avoid coercion within laboratories, I would suggest a bill of labor rights. That is, the principal investigator of the grant have the legal and direct responsibility of coing the actual DNA restriction, mixing, ligating, insertion, and transfection if a plasmid is used; of infection if a phage vector is used. This responsibility should not be an assignable one. This will allow those who do not want to do the experiments, the freedom to decline. The organizational structure of funding at the present time does not allow the freedom to decline. This will also force more careful thought in design of the experiments and hazards.

In summary, my perspective of genotypic selection suggests that the first experiments that should be done are those that test the intrinsic mutability of cloned DNA and the fidelity of its expression. These can be done without hazard or cost. Fortunately, the scientific establishment in the United States has the financial constraint which makes it unlikely that the cheapest experiments will override the more expensive; or the disposable experiment override the capital intensive one.

However, this is, uniquely my perspective, based on a study of naturally occurring nucleotide sequences. The contemporary view of all molecular biologists, especially those that have appeared as witnesses is that the expression of DNA is universal and passive. It is to them a piece of instruction and its expression is unaffected by the mechanism of expression. Time and more research will tell.

As a slight digression, if genotypic selection exists then Donald Frederickson is wrong and a magic bullet for cancer is possible, but that is another story.

Thank you.

Mr. Thornton. Thank you very much, doctor. This is the first time a witness has given a statement more rapidly than I could read it. That is a new experience. I do commend you for a very scholarly presentation. I would like to ask you, with regard to your statement that evolution and not DNA is the basic dogma of life and biology, whether you are tending to exaggerate that by characterizing those studies as dogma.

I personally do not like to assume a dogmatic position. I think that tolerance of views is necessary, that the difference between the Darwinians and the non-Darwinians may not reflect that either is wrong; but that both may be partly right, and partly wrong.

I wonder if you would like to clarify that statement to any degree? Was it an overstatement, or do you think that it is an article of faith?

Dr. Peczynski. Yes. In biological research evolution is an article of faith. You explain phenomena in biochemistry, observations in terms of their adaptiveness. That is why we can say: Why does this molecule exist here? We can ask why questions because we believe that there is a dogma of evolution.

Otherwise we would ask questions of sodium chlorite, table salt. One does not ask why do we have salt. But we can ask why do we have collagen as a structural protein.

Why does DNA consist of four nucleotides? The reason we can ask "why" questions is we live and analyze these molecules as if they belong to the construct developed by Darwin.

Mr. Thornton. Do we ask why they are polypeptides?

Dr. Peczynski. Yes. That is a reasonable question. We are talking about chemicals. That we can ask that question implies that we are asking it within a construct that consists of the dogma of evolution. Scientists don't work within this construct don't ask these questions.

Mr. Thornton. Well, I think perhaps you are using the word "dogma" in somewhat a different—

Dr. Peczynski. It isn't a political dogma.

Mr. Thornton. I suppose, like Lewis Carroll, we will have to arrive at some definition of what the word means, rather than to assume just what either of us intends it to mean.

Dr. Peczynski. There is a concept of evolution called the Red Queen model which comes from "Alice in Wonderland" and it says that, in order for one person to gain, someone else has to lose. Much like in the Red Queen land, you have to run twice as fast to stay where you are.

Mr. Thornton. There are methods which have been suggested which have later been discredited. I believe Lysenko's theories, which set back Russian biological research by many years suggested that acquired characteristics could be inherited. I wondered if a bacteria, E. coli, which acquires a characteristic by genetic manipulation and insertion of genetic information into its structure can pass on that trait. And if so, is Lysenko right but on a different level? Is that an acquired or added trait?

Dr. Peczynski. Lysenko did not believe that DNA was the genetic material. The acquired characteristics were passed on to its bacteria and it becomes adaptive within the bacteria and it survives in the bacteria—

Mr. Thornton. Is that an acquired characteristic for that particular bacteria?

Dr. Peczynski. It is acquired by the bacteria or given to it.

Mr. Thornton. It is inheritable?

Dr. Peczynski. Yes.

Mr. Thornton. In the research which you have done and the testimony which you have brought to our attention, are you operating upon a theory that at least same rules of inheritability, adaptability, survival which apply in gross to organized species also apply at the molecular level?

Dr. Peczynski. That is the idea that I have introduced. That is the idea that I believe is correct and should be tested.

Mr. Thornton. I think it is an interesting concept and I am looking forward to further discussion with the other panels. Mr. Hollerbeck?

Mr. Hollerbeck. Thank you, Mr. Chairman. Doctor, I would like to expand more upon your answer to the chairman's last question. I would like you to address yourself to the statement you made at the beginning of page 4 that at present you do not see the clear and present hazard or benefit from artificial DNA selection.
Is that a conclusion based upon the answer to the chairman's last question?

Dr. Pisczenek. It is a conclusion based on the concept that there is a competition of molecules and also the belief that cloned DNA does not have a competitive advantage over natural DNA in its milieu and the observation, now, that no cloned DNA has had faithful expression and extreme species crosses into a plasmid have had no expression. The hemoglobin gene that was put in a plasmid did not make hemoglobin. So far there is no evidence, and it has been 5 years of experimental work, that there has been faithful or proper expression of the DNA.

That does not point out the fact that small polypeptides—small functions that could have functioned once, isn't happening. Small polypeptides seem to have powerful functions. Things that you don't expect to be made, become the hazard, rot the things you do.

Mr. Thornton. I think it might be useful if we go ahead and hear the other witnesses and then open the hearing to discussion with the entire panel. Can each of you stay aboard for that schedule? I appreciate that.

Next, I would like to introduce and call upon Dr. Robert J. Ryan, the department of molecular medicine of the Mayo Medical School. Dr. Ryan has had a distinguished career and has made some discoveries which may or may not—and I am not sure what the testimony will be or the ultimate outcome will be with regard to that question—be examples of natural recombinant DNA.

We are very pleased to have you with us. We look forward to your testimony.

[A biographical sketch of Dr. Ryan follows.]

DR. ROBERT J. RYAN

Academic rank: Professor.
Mayo appointment: July 1, 1967. G.S. Status: B.
Medical field: I.
Section assignment: MOLECMD 56 A.
Date and place of birth: July 15, 1927, Cincinnati, Ohio.
Retirement date: September 1962.
College/medical school training with degrees and institutions conferring them:
Xavier University, Cincinnati, Ohio, January 1945 to October 1945.
University of Cincinnati, Cincinnati, Ohio, February 1947 to August 1948.
University of Cincinnati, Cincinnati, Ohio, M.D., September 1948 to June 1952.
Internships: Henry Ford Hospital, Detroit, Mich., July 1952 to June 1953.
Residences:
University of Illinois, Chicago Ill., July 1953 to June 1954.
Resident and educational hospitals, Chicago, Ill., July 1954 to June 1955.
Resident and educational hospitals, Chicago, Ill., Chief resident, July 1956 to June 1957.
Tufts University, Boston, Mass., July 1957 to September 1958.
Professional preparation/academic experience:
University of Illinois, instructor in I, July 1956 to June 1957.
University of Illinois, assistant professor of I, January 1968 to September 1962.
University of Illinois, Associate professor of I, September 1963 to July 1967.
Mayo Clinic, consultant in physiology, July 1967 to January 1968.
Mayo Graduate School, associate professor of I, January 1968 to July 1971.
Intraunal activities:
Research committee, vice chairman, 1971.
Building committee, research liaison, 1971.
Molecular medicine, chairman department, July 1974.
Research committee, $750, 1971.