Nobel Lecture

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A Case History in Biological Research

In casting around in search of a new approach, an important consideration was that much of biochemical genetics has been and will be covered by Professor Beadle and Professor Lederberg, and in many symposia and reviews, in which many aspects have been and will be considered in greater detail and with greater competence than I can hope to do here. It occurred to me that perhaps it might be instructive, valuable, and interesting to use the approach which I have attempted to define by the title "A Case History in Biological Research". In the development of this case history I hope to point out some of the factors involved in all research, specifically the dependence of scientific progress: on knowledge and concepts provided by investigators of the past and present all over the world; on the free interchange of ideas within the international scientific community; on the hybrid vigor resulting from cross-fertilization between disciplines; and last but not least, also dependent on chance, geographical proximity, and opportunity. I would like finally to complete this case history with a brief discussion of the present status of the field, and a prognosis of its possible development.

Under the circumstances, I hope I will be forgiven if this presentation is given from a personal viewpoint. After graduating from the University of Wisconsin in chemistry, I was fortunate in having the opportunity of doing graduate work in biochemistry and microbiology at this University under the direction and leadership of W.H. Peterson and E.B. Fred. At that time, in the early 30's, one of the exciting areas being opened concerned the so-called "growth-factors" for micro-organisms, for the most part as yet mysterious and unidentified. I became deeply involved in this field, and was fortunate to have been able, in collaboration with H.G. Wood, then visiting at Wisconsin, to identify one of the required growth-factors for propionic acid bacteria, as the recently synthesized vitamin B1 or thiamine[1]. This was before the universality of need for the B vitamins, and the enzymatic basis of this requirement, had been clearly defined. The vision of Lwoff and Knight had already indicated a correlation of the need of micro-organisms for "growth-factors" with failure of synthesis, and correlated this failure with evolution, particularly in relation to the complex environment of "fastidious" pathogenic micro-organisms.

However, the tendency at this time was to consider "growth-factors" as highly individual requirements, peculiar to particular strains or species of micro-organisms as isolated from nature, and their variation in these respects was not generally considered as related to gene mutation and variation in higher organisms. Actually my ignorance of and naiveté in genetics was probably typical of that of most biochemists and microbiologists of the time, with my only contact with genetic concepts being a course primarily on vertebrate evolution.

After completing graduate work at Wisconsin I was fortunate in being able to spend a year studying at the University of Utrecht with F. Kögl, the discoverer of the growth-factor biotin, and to work in the same laboratory with Nils Fries, who already had contributed significantly in the field of nutrition and growth of fungi.

At this time, Professor Beadle was just moving to Stanford University, and invited me as a biochemist to join him in the further study of the eye-color hormones of *Drosophila*, which he and Ephrussi in their work at the California Institute of Technology and at Paris had so brilliantly established as diffusible products of gene-controlled reactions. During this, my first contacts with modern genetic concepts, as a consequence of a number of factors the observation of Khouvine, Ephrussi, and Chevais[2] in Paris that dietary tryptophan was concerned with *Drosophila* eye-color hormone production; our studies on the nutrition of *Drosophila* in aseptic culture[3]; and the chance contamination of one of our cultures of *Drosophila* with a particular bacterium - we were able to isolate the v^+ hormone in crystalline state from a bacterial culture supplied with tryptophan[4], and with A.J. Haagen-Smit to identify it as kynurenine[5], originally isolated by Kotake, and later structurally identified correctly by Butenandt. It might be pointed out here that kynurenine has since been recognized to occupy a central position in tryptophan metabolism in many organisms aside from insects, including mammals and fungi.

At about this time, as the result of many discussions and considerations of the general biological applicability of chemical genetic concepts, stimulated by the wealth of potentialities among the micro-organisms and their variation in nature with respect to their nutritional requirements, we began our work with the mold *Neurospora crassa*.

I shall not renumerate the factors involved in our selection of this organism for the production of chemical or nutritionally deficient mutants, but must take this opportunity of reiterating our indebtedness to the previous basic findings of a number of investigators. Foremost among these, to B.O. Dodge for his establishment of this *Ascomycete* as a most suitable organism for genetic studies[6];and to C.C. Lindegren[7], who

became interested in *Neurospora* through T.H. Morgan, a close friend of Dodge.

Our use of *Neurospora* for chemical genetic studies would also have been much more difficult, if not impossible, without the availability of synthetic biotin as the result of the work of Kögl[8] and of du Vigneaud[9]. In addition, the investigations of Nils Fries on the nutrition of *Ascomycetes*[10] were most helpful, as shown by the fact that the synthetic minimal medium used with *Neurospora* for many years was that described by him and supplemented only with biotin, and has ordinarily since been referred to as "Fries medium". It should also be pointed out that the experimental feasibility of producing the desired nutritionally deficient mutant strains depended on the early pioneering work of Roentgen, with X-rays, and on that of H.J. Muller, on the mutagenic activity of X-rays and ultraviolet light on *Drosophila*. All that was needed was to put these various facts and findings together to produce in the laboratory with irradiation, nutritionally deficient (auxotrophic) mutant strains of *Neurospora*, and to show that each single deficiency produced was associated with the mutation of a single gene[11].

Having thus successfully tested with *Neurospora* the basic premise that the biochemical processes concerned with the synthesis of essential cell constituents are gene controlled, and alterable as a consequence of gene mutation, it then seemed a desirable and natural step to carry this approach to the bacteria, in which so many and various naturally occurring growth-factor requirements were known, to see if analogous nutritional deficiencies followed their exposure to radiation. As is known to all of you, the first mutants of this type were successfully produced in *Acetobacter* and in *Escherichia coli*[12], and the first step had been taken in bringing the bacteria into the fold of organisms suitable for genetic study.

Now to point out some of the curious coincidences or twists of fate as involved in science: One of the first series of mutants in *Neurospora* which was studied intensily from the biochemical viewpoint was that concerned with the biosynthesis of tryptophan. In connection with the role of indole as a precursor of tryptophan, we wanted also to study the reverse process, the breakdown of tryptophan to indole, a reaction typical of the bacterium *E. coli*. For this purpose we obtained, from the Bacteriology Department at Stanford, a typical *E. coli* culture, designated K-12. Naturally, this strain was later used for the mutation experiments just described so that a variety of biochemically marked mutant strains of *E. coli* K-12 were soon available. It is also of interest that Miss Esther Zimmer, who later became Esther Lederberg, assisted in the production and isolation of these mutant strains.

Another interesting coincidence is that F. J. Ryan spent some time on leave from Columbia University at Stanford, working with *Neurospora*. Shortly after I moved to Yale University in 1945, Ryan encouraged Lederberg, then a medical student at Columbia who had worked some time with Ryan on *Neurospora*, to spend some time with me at Yale University. As all of you know, Lederberg was successful in showing genetic recombination between mutant strains of *E. coli* K-12[13] and never returned to medical school, but continued his brilliant work on bacterial recombination at Wisconsin. In any case, the first demonstration of a process analogous to a sexual process in bacteria was successful only because of the clear-cut nature of the genetic markers available which permitted detection of this very rare event, and because of the combination of circumstances which had provided those selective markers in one of the rare strains of *E. coli* capable of recombination. In summing up this portion of this case history, then, I wish only to emphasize again the role of coincidence and chance played in the sequence of developments, but yet more strongly to acknowledge the even greater contributions of my close friends and associates, Professor Beadle and Professor Lederberg, with whom it is a rare privilege and honor to share this award.

Now for a brief and necessarily somewhat superficial mention of some of the problems and areas of biology to which these relatively simple experiments with *Neurospora* have led and contributed. First, however, let us review the basic concepts involved in this work. Essentially these are: *(1)* that all biochemical processes in all organisms are under genie control; *(2)* that these overall biochemical processes are resolvable into a series of individual stepwise reactions; *(3)* that each single reaction is controlled in a primary fashion by a single gene, or in other terms, in every case a I:I correspondence of gene and biochemical reaction exists, such that *(4)* mutation of a single gene results only in an alteration in the ability of the cell to carry out a single primary chemical reaction. As has repeatedly been stated, the underlying hypothesis, which in a number of cases has been supported by direct experimental evidence, is that each gene controls the production, function, and specificity of a particular enzyme. Important experimental implications of these relations are that each and every biochemical reaction in a cell of any organism, from a bacterium to man, is theoretically alterable by gene mutation, and that each such mutant cell strain differs in only one primary way from the non-mutant parental strain. It is probably unnecessary to point out that these experimental expectations have been amply supported by the production and isolation, by many investigators during the last 15 or more years, of biochemical mutant strains of micro-organisms in almost every species tried: bacteria, yeasts, algae, and fungi.

It is certainly unnecessary for me to do more than point out that mutant strains such as those produced and isolated first in *Neurospora* and *E. coli* have been of primary utility as genetic markers in detecting and elucidating the details of the often exotic mechanisms of genetic recombination of micro-organisms.

Similarly, it seems superfluous even to mention the proven usefulness of mutant strains of micro-organisms in unraveling the detailed steps involved in the biosynthesis of vital cellular constituents. I would like to list, however, a few of the biosynthetic sequences and biochemical interrelationships which owe their discovery and elucidation largely to the use of biochemical mutants. These include: the synthesis of the aromatic amino acids via dehydroshikimic and shikimic acid [14, 15], by way of prephenic acid to phenylalanine[16], and by way of anthranilic acid, indole glycerol phosphate[17], and condensation of indole with serine to give tryptophan[18]; the conversion of tryptophan via kynurenine and 3-OH anthranilic acid to niacin[19, 20]; the biosynthesis of histidin[21]; of isoleucine and valine via the analogous di-OH and keto acids[22]; the biosynthesis of proline and ornithine from glutamic acid[23]; and the synthesis of pyrirnidines via erotic acid[24].

If the postulated relationship of gene to enzyme is correct, several consequences can be predicted. First, mutation should result in the production of a changed protein, which might either be enzymatically inactive, of intermediate activity, or have otherwise detectably altered physical properties. The production of such proteins changed in respect to heat stability, enzymatic activity, or other properties such as activation energy, by mutant strains has indeed been demonstrated in a number of instances[25-31]. Recognition of the molecular bases of these changes must await detailed comparison of their structures with those of the normal enzyme, using techniques similar to the elegant methods of Professor Sanger. That the primary effect of gene mutation may be as simple as the substitution of a single amino acid by another and may lead to profound secondary changes in protein structure and properties has recently been strongly indicated by the work of Ingram on hemoglobin[32]. It seems inevitable that induced mutant strains of microorganisms will play a most important part in providing material for the further examination of these problems.

A second consequence of the postulated relationship stems from the concept that the genetic constitution defines the potentialities of the cell, the time and degree of expression of which are to a certain extent modifiable by the cellular environment. The analysis of this type of secondary control at the biochemical level is one of the important and exciting new areas of biochemistry. This deals with the regulation and integration of

biochemical reactions by means of feed-back mechanisms restricting the synthesis or activities of enzymes[33-36] and through substrate induced biosynthesis of enzymes[37]. It seems probable that some gene mutations may affect biochemical activities at this level, (modifiers, and suppressors), and that chemical mutants will prove of great value in the analysis of the details of such control mechanisms.

An equally fascinating newer area of genetics, opened by Benzer[38] with bacteriophage, is that of the detailed correlation of fine structure of the gene in terms of mutation and recombination, with its fine structure in terms of activity. Biochemical mutants of micro-organisms have recently opened this area to investigation at two levels of organization of genetic material. The higher level relates to the genetic linkage of non-allelic genes concerned with sequential biosynthetic reactions. This has been shown by Demerec and by Hartman in the biosynthesis of tryptophan and histidine by *Salmonella*[39].

At a finer level of organization of genetic material, the biological versatility of *Neurospora* in forming heterocaryotic cells has permitted the demonstration[40-42] that genes damaged by mutation in different areas, within the same locus and controlling the same enzyme, complement each other in a heterocaryon in such a way that synthesis of enzymatically active protein is restored, perhaps, in a manner analogous to the reconstitution of ribonuclease from its a and b constituents, by they production in the cytoplasm of an active protein from two gene products defective in different areas. This phenomenon of complementation, which appears also to take place in *Aspergillus*[43], permits the mapping of genetic fine structure in terms of function, and should lead to further information on the mechanism of enzyme production and clarification of the role of the gene in enzyme synthesis.

The concepts of biochemical genetics have already been, and will undoubtedly continue to be, significant in broader areas of biology. Let me cite a few examples in microbiology and medicine.

In microbiology the roles of mutation and selection in evolution are coming to be better understood through the use of bacterial cultures of mutant strains. In more immediately practical ways, mutation has proven of primary importance in the improvement of yields of important antibiotics - such as in the classic example of penicillin, the yield of which has gone up from around 40 units per ml of culture shortly after its discovery by Fleming to approximately 4,000, as the result of a long series of successive experimentally produced mutational steps. On the other side of the coin, the mutational origin of antibiotic-resistant micro-organisms is of definite medical significance. The therapeutic use

of massive doses of antibiotics to reduce the numbers of bacteria which by mutation could develop resistance, is a direct consequence of the application of genetic concepts. Similarly, so is the increasing use of combined antibiotic therapy, resistance to both of which would require the simultaneous mutation of two independent characters.

As an important example of the application of these same concepts of microbial genetics to mammalian cells, we may cite the probable mutational origin of resistance to chemotherapeutic agents in leukemic cells[44], and the increasing and effective simultaneous use of two or more chemotherapeutic agents in the treatment of this disease. In this connection it should be pointed out that the most effective cancer chemotherapeutic agents so far found are those which interfere with DNA synthesis, and that more detailed information on the biochemical steps involved in this synthesis is making possible a more rational design of such agents. Parenthetically, I want to emphasize the analogy between the situation in a bacterial culture consisting of two or more cell types and that involved in the competition and survival of a malignant cell, regardless of its origin, in a population of normal cells. Changes in the cellular environment, such as involved in chemotherapy, would be expected to affect the metabolic efficiency of an altered cell, and hence its growth characteristics. However, as in the operation of selection pressures in bacterial populations, based on the interaction between cell types, it would seem that the effects of chemotherapeutic agents on the efficiency of selective pressures among mammalian cell populations can be examined most effectively only in controlled mixed populations of the cell types concerned.

In other areas in cancer, the concepts of genetics are becoming increasingly important, both theoretically and practically. It seems probable that neo-plastic changes are directly correlated with changes in the biochemistry of the cell. The relationships between DNA, RNA, and enzymes which have evolved during the last few decades, lead one to look for the basic neoplastic change in one of these intimately interrelated hierarchies of cellular materials.

In relation to DNA, hereditary changes are now known to take place as a consequence of mutation, or of the introduction of new genetic material through virus infection (as in transduction) or directly (as in transformation). Although each of these related hereditary changes may theoretically be involved in cancer, definite evidence is available only for the role of viruses, stemming from the classic investigations of Rous on fowl sarcoma[45]. At the RNA level of genetic determination, any one of these classes of change might take place, as in the RNA containing viruses, and result in an heritable change, perhaps of the cytoplasmic type,

semi-autonomous with respect to the gene. At the protein level, regulatory mechanisms determining gene activity and enzyme synthesis as mentioned earlier, likewise provide promising areas for exploration.

Among the many exciting applications of microbial-genetic concepts and techniques to the problems of cancer, may I mention in addition the exploration by Klein[46] of the genetic basis of the immunological changes which distinguish the cancer cell from the normal, and the studies on the culture, nutrition, morphology, and mutation of isolated normal and malignant mammalian cells of Puck[47] and of Eagle[48]. Such studies are basic to our exploration and to our eventual understanding of the origin and nature of the change to malignancy.

Regardless of the origin of a cancer cell, however, and of the precise genetic level at which the primary change takes place, it is not too much to hope and expect eventually to be able to correct or alleviate the consequences of the metabolic defect, just as a closer understanding of a heritable metabolic defect in man permits its correction or alleviation. In terms of biochemical genetics, the consequences of a metabolic block may be rectified by dietary limitation of the precursor of an injurious accumulation product, aromatic amino acids in phenylketonuria; or by supplying the essential end product from without the cell, the specific blood protein in hemophilia, or a specific essential nutrient molecule such as a vitamin.

Time does not permit the continuation of these examples. Perhaps, however, I will be pardoned if I venture briefly on a few more predictions and hopes for the future.

It does not seem unrealistic to expect that as more is learned about control of cell machinery and heredity, we will see the complete conquering of many of man's ills, including hereditary defects in metabolism, and the momentarily more obscure conditions such as cancer and the degenerative diseases, just as disease of bacterial and viral etiology are now being conquered.

With a more complete understanding of the functioning and regulation of gene activity in development and differentiation, these processes may be more efficiently controlled and regulated, not only to avoid structural or metabolic errors in the developing organism, but also to produce better organisms.

Perhaps within the lifetime of some of us here, the code of life processes tied up in the molecular structure of proteins and nucleic acids will be

broken. This may permit the improvement of all living organisms by processes which we might call biological engineering.

This might proceed in stages from the *in vitro* biosynthesis of better and more efficient enzymes, to the biosynthesis of the corresponding nucleic acid molecules, and to the introduction of these molecules into the genome of organisms, whether via injection, viral introduction into germ cells, or via a process analogous to transformation. Alternatively, it may be possible to reach the same goal by a process involving directed mutation.

As a biologist, and more particularly as a geneticist, I have great faith in the versatility of the gene and of living organisms in providing the material with which to meet the challenges of life at any level. Selection, survival, and evolution take place in response to environmental pressures of all kinds, including sociological and intellectual. In the larger view, the dangerous and often poorly understood and controlled forces of modern civilization, including atomic energy and its attendant hazards, are but more complex and sophisticated environmental challenges of life. If man cannot meet those challenges, in a biological sense he is not fit to survive.

However, it may confidently be hoped that with real understanding of the roles of heredity and environment, together with the consequent improvement in man's physical capacities and greater freedom from physical disease, will come an improvement in his approach to, and understanding of, sociological and economic problems. As in any scientific research, a problem clearly seen is already half solved. Hence, a renaissance may be foreseen, in which the major sociological problems will be solved, and mankind will take a big stride towards the state of world brotherhood and mutual trust and well being envisaged by that great humanitarian and philanthropist Alfred Nobel.

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