CURRENTS IN BIOCHEMICAL RESEARCH

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Edited by DAVID E. GREEN

Thirty-one essays charting the present course of Biochemical Research and considering the intimate relationship of biochemistry to medicine, agriculture and social problems

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PREFACE

With the ever-increasing degree of specialization in scientific research and with the terrifying rate of growth of technical nomenclature, men of science are literally compelled to know more and more about less and less. The scientific literature furthers this trend, since journals, textbooks (apart from those for students), and review articles are written primarily for the specialist. There is an acute need for stripping complex subjects and getting at the simple, essential concepts which are basic to their appreciation. After all, the same scientific principles are applicable to all fields of inquiry. The art of presentation consists in the elimination of the barriers of terminology which effectively conceal these fundamental principles. *Currents in Biochemical Research* represents an attempt by some thirty research workers to describe in as simple language as possible the important developments in their own fields and to speculate a little on the most likely paths of future progress. The aim of these essays has been to excite the imagination and to provide glimpses of some of the fascinating horizons of biochemical research. However, no popularizations were intended. The various contributors were asked to write simply and provocatively but without sacrifice of scholarship. Dealing as they do on the one hand with pharmacology, chemotherapy, public health, genetics, photosynthesis, and agriculture and on the other with considerations of organic, analytical, and physical chemistry, they emphasize the focal position of biochemical research in biology, chemistry, and medicine. It is hoped that this survey from so many different points of view may assist biochemists, chemists, and medical doctors in seeing biochemistry in clearer perspective and in its proper relation to other fields of inquiry.

DAVID E. GREEN

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CONTENTS

CONTENTS

1

THE GENE AND BIOCHEMISTRY

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IT IS both an accident of organic evolution and an indication of man's lack of foresight that the organisms studied in most detail by biochemists have not been those on which geneticists have concentrated. It is natural that man should have a prejudice in favor of himself, and it is therefore not remarkable that the urge of medicine on biochemistry has been in the direction of specialization on mammals, particularly on man himself. For obvious reasons, bacterial biochemistry has likewise been well nourished through medicine. Man has few inherent advantages for biochemical study while the bacteria abound in them. But both are most difficult for the geneticist—the one because of a long life cycle and social obstacles to controlled matings, the other because of the absence of a sexual cycle without which the geneticist cannot use his particular methods. The geneticist, on the other hand, has chosen to make the vinegar fly and Indian corn the classical organisms of his science. Both suffer disadvantages to the biochemist in not lending themselves readily to culture under precisely defined environmental conditions. Neither can be grown conveniently on a medium completely known from a chemical standpoint.

In spite of this situation and additional impediments arising through divergence in outlook, such persons as Garrod, Onslow (née Wheldale), Troland, Goldschmidt, Wright, Haldane, and others have

urged that the two fields have much in common and that each stands to profit through contact with the other. Through the efforts of these individuals and others of like mind there are many instances known in which the relation of genetics to biochemistry is so clear that it can no longer be disregarded by intelligent investigators in either field. In fact, from this relation there tends to emerge a new interest, known as biochemical genetics, which promises to tell us what the genes do and how they do it, on the one hand, and to lead us to further knowledge in the ways of biosynthesis on the other. In both directions there obviously lie many opportunities.

One of the earliest instances in which a Mendelian trait could be interpreted in terms of specific chemical reactions is that involving the human disease known as alcaptonuria. In individuals homozygous for the mutant gene responsible for this character, 2,5-dihydroxyphenylacetic acid (homogentisic acid or alcapton) is excreted in the urine instead of being broken down to carbon dioxide and water, as it is in persons receiving the normal form of the alcaptonuric gene from one or both parents (15). Homogentisic acid is oxidized to a black pigment on exposure to air and it is this process that is responsible for darkening of the urine, the most striking symptom of the disease. According to Gross (cited by Garrod), alcaptonurics lack a specific enzyme found in the blood of normal persons which catalyzes the degradation of homogentisic acid. Alcaptonuria therefore represents the first recorded instance in which it could be said that a particular chemical reaction is controlled by a known gene through the mediation of a specific enzyme.

Within the past dozen years, additional examples have become known in which organisms unable to carry out specific reactions differ in a single gene from their chemically more successful relatives. In flower pigment synthesis, for example, the formation of carotenoids, anthocyanins, anthoxanthins, chalcones, and flavocyanins is known to be genetically controlled in one plant or another (7,24). Specific oxidations of pelargonidin derivatives to cyanidin analogues and of cyanidin compounds to delphinidin counterparts are dependent on the activities of specific genes. The addition of sugars to anthocyanidins through glycosidal linkages and the transformation of the anthoxanthin quercetin-3-glucoside to the corresponding cyanidin-3-glucoside are likewise unable to proceed if specific genes are modified.

Fölling (14) and Penrose (35) have shown that the genetically determined failure to oxidize phenylpyruvic acid in man is invariably associated with subnormal mentality. Here again there appears to be an intimate relation between a particular gene and a specific chemical reaction. Because of its obvious importance to an understanding of the mechanisms underlying mental processes, this case is of particular interest. It is of course related metabolically to alcaptonuria in so far as phenylalanine is concerned in both. Other abnormalities in phenylalanine–tyrosine metabolism are also known (15,19).

Most remarkable progress has recently been made in understanding the genetic and chemical mechanisms of sex determination and differentiation in the green alga, *Chlamydomonas*, by Moewus, Kuhn, and co-workers. From the carotenoid pigment, protocrocin, there is derived through cleavage the motility hormone, crocin, and a female-determining hormone known as gynotermone. This cleavage is known to be genetically controlled. In genetically male individuals gynotermone is hydrolyzed to a male-determining hormone known as androtermone. Under the direction of specific genes the *cis* and *trans* forms of the motility hormone, crocin, are converted into the corresponding *cis* and *trans* dimethyl esters of crocetin. In various specific mixtures, these serve as gamones, *i. e.*, they render individuals of the specific genetic constitutions capable of conjugation. The relations between genes and chemical reactions disclosed by this work support the thesis that genes act in directing specific processes. The work on *Chlamydomonas* is so spectacular and its importance so great that independent confirmation is desirable (see 7,28,41).

The splitting of specific di- and trisaccharides by yeasts is under genetic control, as shown by Winge and Laustsen (54) and Lindegren, Spiegelman, and Lindegren (25). It appears that the genes concerned determine whether or not specific enzymes are present in active form. Somewhat similar situations are known in the rabbit, where Sawin and Glick (37) have shown that the activity of the enzyme, atropine esterase, is dependent on the presence of the normal allele of a particular gene, and in white clover, where an enzyme responsible for hydrolysis of specific cyanogenetic glucosides is known to show a similar dependence on a gene (2,9).

In the bread mold, *Neurospora*, Srb and Horowitz (44) have shown that there is present an ornithine cycle essentially similar to

that postulated by Krebs and Henseleit for the mammalian liver. In the bread mold it is known that mutation in any one of seven different genes will interrupt the synthesis of ornithine or its conversion to arginine. So far as the data go, they are consistent with the assumption that each of the seven genes is normally concerned with a different chemical reaction in the system. It is an interesting point that it was possible to establish the presence of the ornithine cycle in *Neurospora* because of the existence of the mutant strains indicated.

Tatum and Bonner (50) have shown that tryptophan is normally synthesized in *Neurospora* through the condensation of indole and serine. Evidently the indole is somehow derived from anthranilic acid, for there exist two mutant strains, one of which accumulates anthranilic acid when it is grown under suitable conditions, while the other is able to grow normally when supplied with anthranilic acid in place of indole or tryptophan (51). The gene by which the first strain differs from wild type is evidently concerned with the reaction by which anthranilic acid is converted into indole, whereas the mutant gene of the second strain appears to be concerned with failure of some reaction essential to the synthesis of anthranilic acid. It is obvious, in this case, that genetics has provided a tool of great usefulness in investigating the biosynthesis of the important amino acid, tryptophan.

Relations similar to those mentioned above are known for other biosyntheses in the bread mold and in other organisms. By following methods developed by Beadle and Tatum (8), it has been possible to obtain a series of mutant strains of *Neurospora* in each of which some particular reaction has been blocked. These are concerned with the synthesis of amino acids, vitamins, purines, pyrimidines, and other compounds of biological importance (6,21,48,49).

We can be sure from such cases as those just cited that genes function in directing biochemical reactions. We know, further, that this direction may involve enzymes as intermediates between gene and reaction. All our information is consistent with the hypothesis that in all cases in which genes control specific reactions they do so indirectly through enzymes. In other words, genes direct enzyme specificities, and enzymes control reactions. This is not a new idea. Bateson (4), Moore (29), Troland (52), Goldschmidt (16), Muller (30), Alexander and Bridges (1), Haldane (18,19), Wright (55), and others have sug-

gested it. We are only now beginning to do something definite about it from an experimental standpoint. Since the specificities of enzymes are referable to protein specificities, the hypothesis implies that genes direct protein specificities. In this case we might expect that the specificities of proteins other than those found in enzymes would show a direct relation to genes. This is indeed the situation as evidenced by the fact that in many organisms a general one-to-one relation between genes and antigens has been shown (22,47). It is true that a few deviations from this correspondence are known, but they may well represent instances in which antigens have specificities made up of two components, each corresponding to one gene.

If we knew the chemical nature of genes, we should be in a much better position than we are now to determine how they direct protein specificities. Direct chemical analyses of whole chromosomes show them to be largely nucleoprotein (27), which suggests that genes too are nucleoproteins. But since chromosomes probably contain much nongenic material, the deduction is not too satisfying. Ultraviolet radiation induces gene mutation; and its efficiency in this respect varies with wave length in the same way as does its absorption by nucleic acid (20,45), strongly indicating that the energy effective in producing mutations in genes is absorbed by nucleic acid. The simplest assumption possible is that this is so because the nucleic acid is part of the gene.

The similarity of genes and viruses constitutes a third line of evidence concerning the chemical nature of genes. Both have the property of self-duplication, which in both cases is dependent on the presence of a series of compounds such as those found in the living cell. Genes and viruses appear to be within the same size range (46). Both are capable of undergoing mutation to new forms which have altered biological activities but retain the power of self-duplication (46). Since viruses and genes have so many properties in common, it is probable that they are similar in chemical makeup. Following Stanley's isolation of crystalline tobacco mosaic virus, several other viruses have been prepared in pure form and all have been shown to be nucleoproteins (5,12,46). The circumstantial evidence that genes, too, are nucleoproteins, or at least contain nucleoproteins as essential parts, is therefore substantial.

In duplicating themselves, genes have been assumed to act as

master molecules or models from which exact copies are made (11,18, 19,30,31,55). If this is so, their action may be visualized as one of directing the construction of specific protein types plus whatever other component parts genes may have. If the specificities of proteins generally are copied from genes, the observed relations between genes and enzymes and between genes and antigens should follow. For every specific protein there should exist a gene carrying this same protein. For every enzyme and antigen type there likewise should be a gene. Because of a general tendency of mutation pressure to eliminate genes that are of no advantage to the organisms, it might be expected that for every protein type there would be only one corresponding gene. The experimental evidence appears to support this general interpretation, although it must be recognized that, in dealing with genetic traits that can be described in terms of chemical reactions, there may be an unavoidable selection of those cases in which gene action is relatively simple.

However proteins and other components of genes are synthesized —whether by an orthodox stepwise mechanism or by some as yet unknown mechanism by which many component parts are simultaneously directed into their proper places by the master molecule (11)—the precursors of proteins, nucleic acids, and whatever other parts genes may have, must be synthesized. Their synthesis will involve many enzymes and a corresponding number of genes. Thus, before one gene can determine the specificity of a new protein molecule, many other genes must have acted. This amounts to saying that, in any multigenic organism, the genes constitute a highly organized system, just as the chemical reactions they direct are integrated in time and space in a manner characteristic of a particular species. Furthermore, while a particular gene will have only one primary action in determining specificity of an enzyme or an antigen, the final physiological consequences of a change in a single gene will be manifold. This can be appreciated when one considers the consequences of depriving an organism such as a rat of thiamin. The final consequence is of course death, but before death occurs a series of changes of increasing complexity take place. These can be brought about in the rat by removing thiamin from the diet. In the bread mold, which normally synthesizes thiamin, the same end result can be effected as a result of an analogous series of changes initiated by replacing a normal gene

necessary for thiamin synthesis with a defective form of the same gene. In one particular case, the primary action of the gene is presumed to be in directing the specificity of the enzyme catalyzing the reaction by which thiazole and pyrimidine are combined (49). Viewed in this way, an understanding of gene action does not appear hopelessly difficult even though the final effects of a single gene change may involve alterations so complex as to defy complete description. Grüneberg (17) has pointed out that a similar type of interpretation in terms of one primary action of a given gene is tenable in the case of certain hereditary developmental defects in the mouse and rat that at first sight appear to involve several unrelated changes in the organism. That the gene has a functional as well as structural unity is therefore a hypothesis that has demonstrated its heuristic value. Until evidence with which it is inconsistent is presented, it will no doubt continue to play an important role in our concepts of what the gene is and how it acts.

As Troland (52), Muller (30), Alexander and Bridges (1), Oparin (33), Plunkett (36), and others have pointed out, the similarity of viruses and genes suggests that the first living structures, *i. e.*, those with the power of self-duplication, were probably somewhat similar to present-day viruses with the important difference that they were free-living. The evolution of systems of such units, each acquiring the property of directing the specificity of an enzyme or other protein, would be expected to give rise to a series of forms of increasing complexity such as we see today in the larger and more complex viruses, the rickettsias, bacteria, and higher organisms. It is probable that the present viruses and rickettsias are not relics of these ancestral forms but are forms secondarily derived through specialization in connection with parasitism (10). The true ancestral types must have been capable of multiplying outside living cells in a kind of environment which, because of the presence of many organisms, is no longer likely to exist (33). In terms of genes directing chemical reactions through their control of enzyme specificities it is possible to imagine how, in principle, these simple forms evolved in the direction of the more highly specialized and complex forms of multicellular plants and animals (56), although it is of course not easy to visualize the way in which the process occurred in detail in particular instances.

In the specialization of higher animals with respect to their nutri-

tion, it is possible to suggest a scheme of evolution that has some support at least in analogy. It has become increasingly evident that, with respect to their need for and use of vitamins of the B group, purines, pyrimidines, choline, amino acids, and other compounds, all cellular organisms are fundamentally very similar (23,26,53). To consider a specific example, carboxylase is presumably present in all protoplasm and apparently always contains thiamin as thiamin pyrophosphate. Many organisms, *e. g.*, most plants, are able to synthesize the thiamin they need, while others are dependent on an external supply of this essential compound. From an evolutionary standpoint, this difference is presumably determined by whether or not it is of advantage to a particular organism to synthesize thiamin. Evidently for *Neurospora* there is selective advantage in being able to carry out this synthesis for we find in wild strains that all essential genes concerned with it are present in active form. In mammals, on the other hand, thiamin is presumably so frequently present in the diet that the genes originally concerned with its elaboration have been permitted by natural selection to become inactive so far as thiamin synthesis goes. It may well be that they have not disappeared entirely but have been modified so as to enable the mammal to carry out chemical reactions of which the bread mold is incapable. In a similar way, mammals have become specialized through loss of ability to synthesize other vitamins, the indispensable amino acids, and other compounds. With the development of parasitism it would be expected that still further loss in synthetic ability would be encountered. As Knight (23), Lwoff (26), Schopfer (38), and others have pointed out, this is indeed the case. The work on *Neurospora* makes it most probable that the dropping out of specific chemical reactions no longer of selective advantage is the result of gene mutation. The limit of such parasitic specialization is probably represented in the molecular viruses that have lost all power of heterosynthesis and have retained only the one property essential for their continued existence in an environment in which all necessary compounds are available—the property of autosynthesis.

One may quite properly raise the question as to the course of positive evolution in terms of chemical reactions—how are new syntheses developed in the course of organic evolution? Unfortunately, the experimental evidence bearing on this is meager, which is not surprising, for obviously it should be much easier to destroy or inactivate a

complex self-duplicating unit than to modify it so as to give it a new and useful property without sacrificing its power of self-duplication. The first self-duplicating unit must have evolved from nonliving matter at some time, and more complex forms must have evolved from it—the alternative is some form of special creation. There would seem to be less difficulty in imagining a primitive "protogene" mutating to a true gene with a heterocatalytic property than its spontaneous origin in the first place, even if, as Oparin (33) supposes, it arose in a world containing preformed organic molecules of many kinds. Nor is there any apparent reason why such protogenes could not mutate in many different directions in order to give rise to many different organic catalysts. In present-day cellular organisms there exists a possible mechanism for acquiring totally new reactions. Occasionally, through accident, one or more genes become duplicated, *i. e.*, a small segment of a chromosome occurs twice in every set. The duplicated genes will be unnecessary to the organism and will be expected to disappear through loss mutations, since such mutations are not disadvantageous. But such a duplicate gene may occasionally undergo mutation in such a way that it directs the formation of an entirely new enzyme. If this new enzyme should happen to catalyze a reaction that improved the organism in competition with its relatives, the new reaction would be retained. Such new reactions might add new compounds or they might bring about the reverse of the specialization process, which leads in the direction of parasitism. In this way, as Horowitz (20a) has pointed out, the first primitive organisms might gradually have built up systems of synthesis which freed them of their dependence on preformed organic molecules originally present in the environment.

Through such advances as have been indicated we appear to be moving rapidly in the direction of a better understanding of what genes are and what they do. We are no longer content with a knowledge of the laws by which they are transmitted from one generation to the next. We see that they are basic functional units of the organism and that, by taking advantage of their tendency to mutate, we can use them as powerful tools in determining the course of biosynthesis and in understanding other aspects of metabolism. Their relations to enzymes and antigens are becoming known. Precisely how they function in duplicating themselves and in directing the specificities of proteins, nucleic acids, and possibly other large molecules is a question

for the future. But there can be no doubt that the years that lie ahead will be exciting ones in this field. The work of Avery and his co-workers (3) on the transformation of types in *Pneumococcus* and that of Emerson (13) and of others suggests that we may one day learn to direct gene mutations in predetermined ways. Work on enzymes (32) and viruses (5,46) is so closely related to the general problem of gene structure and gene action that only short steps appear to be necessary to bridge the gaps that separate them. Nucleic acid certainly plays an important role in gene action and in protein synthesis (34,39,40), and it is not too much to hope that this role will be made clear in the near future. The relation of genes to cytoplasmic elements is not well understood, but after many years in which discouragingly little progress has been made, important leads are being followed by Sonneborn (42), Spiegelman (43), and others. After half a century of growth, genetics seems to be assuming a position in the broad field of biology in which its close relations to evolution, development, physiology, and biochemistry are now more evident.

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