

THE FUTURE OF ENZYME RESEARCH *

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I am honored to have been invited to speak at this great International Symposium on Enzymes, and especially to be allowed to give a talk with the title The Future of Enzyme Research. This title suggests that I think that I know enough about the subject of enzymes to extrapolate its course into the future - I do not - or that I have a scientific crystal ball that I can look into - I do not. What I do have is a deep belief about the importance of enzymes as subjects for investigation, and a sincere admiration for enzyme chemists and biologists; and I am happy to be associated with them, even if only in the peripheral capacity of an after-dinner speaker.

The problem of enzymes encompasses essentially the whole of biology. When we understand enzymes - their structure, the mechanism of their synthesis, the mechanism of their action - we shall understand life, except for those aspects of life that involve mental processes; and I have no doubt that enzymes are important for these too.

Enzymes do an extraordinary job - that of causing chemical reactions to take place in the body, at body temperature, which without enzymes can be made to take place only under much different conditions or with great difficulty. The outstanding characteristic of enzymes is their specificity. For example, the enzyme β -galactosidase is able easily to hydrolyse a galactoside, whereas it has little effect on the hydrolysis of a glucoside, which differs from the galactoside only in the spatial configuration about a single carbon atom.

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The specificity that is shown by enzymes in their activity is not unique to these substances. It is found also in the ability of antigens to produce specific antibodies, in the action of drugs and hormones, and in the action of genes in producing replicas of themselves and in serving also to produce other substances of well-defined structure. Of the various molecules that demonstrate specificity in their physiological activity, genes are the best. Antibodies are heterogeneous - they have the power of reacting not only with the antigen that caused their manufacture, but also with other antigens, more or less closely similar to the original one; enzymes may serve to catalyze reactions involving several different, somewhat related, substances; but genes seem to be nearly perfect in their action.

It is easy to understand why genes come closer to perfection than the other molecules with specific biological activity - there is an automatic mechanism for making genes better.¹ The manufacture of an antibody molecule is a process that occurs only once, so far as that molecule is concerned. I think that in the manufacture of an antibody molecule the antigen serves as the template, the antibody being molded about it. If the conditions under which the antibody molecule is being manufactured be such as to favor the formation of a good molecule, with high complementariness to the antigen molecule, the resulting molecule will be highly specific in its activity - it will be able to combine very strongly with the homologous antigen, and much less strongly with other molecules; but if conditions are unfavorable (including the operations of chance during the process of synthesis of the molecule) the antibody molecule that is formed may have only small power of combining with this antigen, and a greater power of combining with other molecules. There is no process of selection that operates to improve the

quality of the antibody molecules that are manufactured with use of the injected antigen as the template. The process of duplication of genes is different in nature: it permits a search to be made for a molecular structure with improved power of reduplication. Let us consider, as an example, a gene that has undergone mutation. This molecule, which we may call A_1 , serves as a template for the manufacture of a complementary molecule, B_1 . This molecule, B_1 may then serve as the template for the manufacture of another ^{molecule,} A_2 , complementary to B_1 and similar to A_1 . By repetition of this process the series of molecules, each complementary to the preceding one, is continued: B_2, A_3, B_3, A_4, B_4 . If at any time in this process a molecule A_n happens to be formed that is perfectly suited to serving as ^{the template in} the two-stage manufacture of a duplicate of itself, the process of duplication from then on would be a perfectly reliable one. In this way, the mechanism of heredity permits the ultimate discovery of a gene with the power of duplicating itself perfectly; or perhaps of a gene with such a structure that the process of duplication, if not perfect, is quickly self-correcting.

In the above discussion we have assumed that biological specificity is the result of complementariness in structure, rather than identity in structure. Each of these two alternatives has its appeal. The idea that there are special forces that operate between identical molecules has been suggested to a number of investigators by the resonance phenomenon in quantum mechanics. It seems likely that the specific forces resulting from this phenomenon, operating between identical molecules, are so weak as to be negligible, in the case of large molecules such as proteins and nucleic acids.² There is no doubt whatever that the specificity of the interaction of antibodies and antigens is due to a detailed complementariness in surface configuration and structure that permits the antibody molecule and the antigen molecule to come

into close contact (to within about 1 \AA of the minimum distance of approach) over a surface area of approximately 100 \AA^2 , and that the integrated weak forces of van der Waals attraction, coulomb attraction between positively charged and negatively charged groups, and hydrogen-bond formation between complementary hydrogen donors and acceptors leads to a specific bond of significant strength between the complementary molecules. I think that complementariness in molecular structure of the same sort is responsible for biological specificity in general. In particular, I think that it is likely that many enzymes - perhaps all enzymes except those involved in the transfer of electrons during oxidation-reduction reactions - are effective because of a complementariness in structure to the reacting molecules. By the "reacting molecules" I do not mean the substrate molecules themselves, with their normal configurations. If an enzyme were to have a structure complementary to that of the substrate/^{molecules}in their normal configurations, the combination of the substrate molecule with the enzyme would stabilize the system, and in this way would increase the energy of activation associated with the reaction, and hence decrease the speed of the reaction. If, however, the enzyme were complementary in structure to the activated complex - the half-way structure in the chemical reaction - and not to the reactant molecules or the product molecules, then the energy of interaction of the enzyme with the activated complex would decrease the energy of activation, and hence increase the speed of the reaction. This is a reasonable picture of enzymic activity, and one that is supported by experimental information about inhibition of enzyme reactions. We see that a molecule of an excellent inhibitor of an enzyme is one that simulates in its structure the activated complex. By the continued study of inhibition of enzyme reactions and determination of the detailed molecular structure of the inhibitors it may be possible

to reach some reliable and detailed conclusions about the structure of the enzyme itself, in the region carrying the activity.

In order to increase our knowledge of the structure and mechanism of action of enzymes more work must be done in the field of molecular structure. In particular, the present structure theory of chemistry, which applies in the main to substances in their normal states, must be extended to cover also the activated complexes. Because of the brief period of time during which the activated complexes exist, in the course of chemical reactions, the experimental study of their structure is difficult. I think that it is likely that the development of the field of structural chemistry of activated complexes will be made in large part through the application of theory - an extrapolation from the chemical structure theory of substances in their normal states.

Enzymology as a science now stands where organic chemistry stood a century ago, before the structural formulas of chemistry ^{were} developed. In the course of the many interesting papers about enzymes that were presented at the symposium today there was not shown a single lantern slide giving the interatomic interrelationships of an enzyme and the activated complex for the catalyzed reaction. The time is rapidly approaching when enzyme chemists will use structural formulas of activated complexes, and structural formulas of the enzymes with all interatomic dimensions correct; when this time comes, enzymology will have reached the stage in its development that has now been reached by organic chemistry.

Enzymes are proteins. During recent years we have seen the astounding development of chemical methods, including chromatography, to such power as to permit the determination of long sequences of amino acids in the polypeptide chains of proteins. It is evident, however, that an understanding of the specific properties of proteins cannot be obtained through knowledge of amino-acid sequences alone. It will also be necessary to have knowledge of the way in which the polypeptide chain is folded, and the detailed configuration in space of the side chains. I am

confident that the x-ray investigation of crystals of globular proteins will be successful in leading to the complete structure determination - the location of every atom - of the molecules of many proteins long before the next fifty years of enzyme research is over. I am willing to forecast that within ten years there will have been made such a complete structure determination of at least one crystalline globular protein.

It is hard to forecast to how great an extent enzyme research during the next fifty years will lead to progress in the field of medicine. It is now known that a number of diseases are the result of some sort of abnormality involving enzymes, and that perhaps these diseases should be described as molecular diseases, resulting from the synthesis of enzymes of abnormal structure, which are not able to carry out in a proper way the work that the enzyme normally does. Sickle cell anemia was the first disease to be recognized as resulting from the gene-controlled synthesis of an abnormal molecule, in this case the hemoglobin molecule. It has been shown that a condition that may be called a disease occurs in *Neurospora* as a result of the manufacture of abnormal molecules of the enzyme tryptophan synthetase. The disease phenylketonuria, which leads to mental deficiency and other manifestations, seems clearly to be the result of a gene-controlled failure of the individual to manufacture an effective enzyme that normally catalyzes the process of oxidation of phenylalanine to tyrosine. Perhaps the genic abnormality is such as to lead to a complete interruption of the process of manufacturing this enzyme, or perhaps an abnormal molecule is manufactured, which does not have the normal enzymic activity. A score of diseases have so far been recognized as enzyme diseases, presumably resulting from the manufacture of abnormal molecules in place of the active enzyme molecules. I think that it is not unlikely that there are hundreds or thousands of such diseases.

I foresee the day when many of these diseases will be treated by the use of artificial enzymes. When our understanding of enzyme activity becomes great enough, it will be possible to synthesize a catalyst for the oxidation of phenylalanine to tyrosine. A small amount of this catalyst may then be attached to a reticular framework inside of a small open-ended polythene tube, which can be permanently placed within an artery of a new-born child who has been shown by the presence of phenylpyruvic acid in the urine to have inherited phenylketonuria; through the action of the catalyst the child should then develop in a normal way. This idea seems fantastic now; but the world of 1955 is a fantastic world from the viewpoint of 1905, and I have little doubt that my prediction about the world 2005 will turn out not^{to} be a bold one, but rather a timid and unimaginative one.

Enzymes are wonderful substances - they consist of wonderful molecules. We do not know very much about these molecules as yet, and so far during this International Symposium on Enzymes there has been very little discussion of the new experimental information about enzymes in relation to the detailed molecular structures of the enzymes and the reacting molecules. I am sure that the field of enzyme research will be much different even fifteen years from now - that there will then be much greater knowledge than we now have of structure and activity of enzymes. I hope that fifteen years from now there will be another international symposium on enzymes here in Detroit, and that I may have the opportunity to attend it - and I predict that many of the speakers at this symposium, 1970, will illustrate their papers by the use of slides showing detailed molecular structures of enzymes and reacting molecules.

¹ L. Pauling in discussion of 'Action of X-rays at low Temperatures on the Genetes of Drosophila, by L. Novitski, Amer. Nat., 83, 189 (1949).

² L. Pauling and M. Delbruck, Nature of the Intermolecular Forces Operative in Biological Processes, Science, 92, 77 (1940).
