THE MOLECULAR BASIS OF GENETICS

by

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I am sure that most mental disease is chemical in origin, and that the chemical abnormalities that are involved are usually the result of abnormalities in the genetic constitution of the individual. I think that it is probable that mental illness often results from a quantitative biochemical abnormality - the presence in the individual of molecules of a substance that is normally present, but in concentrations somewhat larger than normal or somewhat smaller than normal. Presumably, the manufacture or the retention of the substance in too large amount or too small amount is often the result of the genetic constitution of the individual, although in some cases it may be attributed to his environment - for example, to the nature of the food that he eats. On the other hand, mental deficiency seems to be often the result of a qualitative abnormality: the presence in the patient of molecules that differ in their structure from those that are present in a normal human being. The manufacture of abnormal molecules of this sort is determined by the genetic constitution of the patient; the disease is inherited. A disease of this sort, caused by molecules of abnormal structure present in the patient in place of the molecules of normal structure that are present in normal human beings, is called a molecular disease.

The expression molecular disease is here used in a special way. All

human beings are made up of molecules, and in a sense one might say that all diseases involve these molecules, and perhaps also the molecules that make up bacteria and viruses, and that accordingly all diseases are molecular diseases. The restriction of the expression molecular disease to diseases that are due to abnormal molecules, differing somewhat in structure from related molecules that are present in normal human beings, seems to me to be a useful one.

Sickle-cell anemia was the first disease to be shown to be a molecular disease.[1] In this disease the red cells of the blood are twisted out of shape, when they are in the venous circulation - they regain their normal shape in the arteries. The twisted red cells become sticky, they clump together, and sometimes interfere with the flow of blood to some parts of the body and cause damage by anoxia; they are also rapidly removed from the circulation, causing the patient to become anemic. The disease seems to be a disease of the red cell, a cellular disease; however, it was found that in fact the hemoglobin molecules manufactured by the patient are abnormal, differing significantly in their structure and properties from the hemoglobin molecules manufactured by normal individuals, and it is clear that the disease is a disease of the hemoglobin molecule.

Although the complete molecular structure of sickle-cell-anemia hemoglobin is not known, nor, in fact, the structure of normal hemoglobin or any other protein, the way in which the molecular abnormality causes the manifestations of the disease sickle-cell anemia is clearly understood. The molecules of the sickle-cell-anemia hemoglobin have such a structure that they clump on to one another easily, to form long rods, which line up side by side to produce a liquid crystal of the nematic type. As this liquid crystal

grows inside of the red cell, it becomes longer than the diameter of the cell, and in its continued growth it twists the cell out of shape.

There is strong evidence that the combination of the molecules of sickle-cell-anemia hemoglobin with one another is caused by a detailed complementariness in structure of one part of the molecule and another part of the molecule, so that the weak intermolecular forces that operate between protein molecules in general are able to collaborate when the surface region of one molecule comes into juxtaposition with the complementary surface region of a second molecule, forming a bond that holds the molecules tightly together. This complementariness in structure is destroyed when the hemoglobin molecule combines with oxygen, and accordingly the process of sickling of the cells is reversed when the blood is oxygenated.

One of the properties in which sickle-cell-anemia hemoglobin differs from normal adult human hemoglobin is the electric charge: molecules of the two kinds of hemoglobin differ from one another by about three electronic charges. The way in which sickle-cell-anemia hemoglobin was recognized as a substance with different molecular structure from normal adult human hemoglobin was the measurement of the mobility of the two hemoglobins in an electric field, using the Tiselius electrophoresis apparatus. The genetic origin of the molecular abnormality was clearly indicated when the hemoglobin from the parents of a sickle-cell-anemia patient was studied. The hemoglobin of each parent was found to consist of a mixture of approximately equal amounts of the two kinds of hemoglobin. Accordingly, the parents were identified as heterozygotes, containing two allelomorphic genes at some level in a pair of chromosomes. One of these genes manufactures normal adult human hemoglobin, and the other one manufactures sickle-cell-anemia

hemoglobin. The parents are in good health, so far as the anemia is concerned - the dilution of the abnormal hemoglobin by the normal hemoglobin prevents the sickling process from occurring in the heterozygotes, except under unusual conditions, as at very high altitudes, where the partial pressure of oxygen is low. When two of the heterozygotes, the carriers of the sickle-cell-anemia gene, marry, one-quarter of their children may be expected to have the disease sickle-cell anemia, one-quarter to be normal, and one-half to be carriers, like the parents.

The question of the continued high incidence of the sickle-cell-anemia gene, despite its continued loss because of the lethal character of the homozygous condition, has been raised by Neel,[2] who suggested three alternative explanations: (1) continued production of the sickle-cell-anemia allele through mutation; (2) the existence of an abnormal genetic mechanism that favors the heterozygous condition over the normal condition; (3) a positive selection of the heterozygotes, perhaps through increased fertility. The first explanation must be rejected because the rate of mutation that would be required is far greater than any that has ever been observed for any organism. There now exists evidence indicating that the third alternative provides the correct explanation, and that malaria is involved. It was first suggested by Brain[3] that the nature of the red cells in the sickle-cell-anemia carriers might give protection against malaria parasites, and thus confer an advantage that would balance the disadvantage of the lethal homozygosity. A test of the hypothesis was carried out by Allison,[4] who infected fifteen healthy adult Africans with sickle-cell-anemia heterozygosity and fifteen similar healthy adult Africans with normal hemoglobin with Plasmodium falciparum by subinoculation with 15 ml. of

blood containing a large number of trophozoites or by allowing them to be bitten by heavily infected anopheles mosquitoes, in which the presence of sporozoites was confirmed by dissection of the mosquitoes. The infection was established in fourteen out of the fifteen Africans without the sickle-cell-anemia heterozygosity, and in only two of the fifteen normal Africans. It was concluded by Allison that the abnormal erythrocytes of the heterozygous individuals are less easily parasitized by P. falciparum than are normal erythrocytes, and that accordingly those individuals who are heterozygous for the sickle-cell-anemia allele have a selective advantage over normal individuals in regions where malaria is hyperendemic.

It is, of course, not unreasonable that the abnormal hemoglobin might be less effective than normal hemoglobin in nourishing the parasites; moreover, it is known that the parasitized erythrocyte uses up oxygen 100 times as fast as a normal erythrocyte, and it might be expected, as suggested by Allison, that the de-oxygenated erythrocyte would sickle, and thus crush the parasite. Accordingly, we have a molecular mechanism not only for the disease sickle-cell-anemia, but also for the protection that the heterozygous condition provides against malarial infection.

Since the discovery was made of the first abnormal hemoglobin, about ten more have been discovered, and about a dozen diseases, kinds of hereditary hemolytic anemia, have been recognized as caused by these abnormal hemoglobins.

I think that it is likely that many kinds of mental retardation are molecular diseases, caused by the gene-controlled manufacture by the patient of abnormal molecules in place of normal ones that are manufactured by normal individuals. There is strong indication that phenylpyruvic

oligophrenia is a molecular disease, or perhaps a complex of molecular diseases. The investigations of Pölling, Jervis, and others have shown clearly that phenylpyruvic oligophrenia is the result of a homozygous genetic abnormality that affects an enzyme that normally catalyzes the oxidation of the amino acid phenylalanine to tyrosine. The patients with phenylpyruvic oligophrenia are not able to carry out this oxidation effectively; they do not manufacture an effective enzyme to catalyze the reaction. We may infer that the patient has inherited from each of his parents an abnormal gene, which leads to the manufacture of an abnormal molecule in place of the normal enzyme. The alternative is that there is a block in the process of synthesis of the enzyme, so that nothing at all is manufactured - it may not be important to differentiate between the failure to manufacture the enzyme and the ability to manufacture an abnormal molecule that is not able to perform the catalytic function of the enzyme.

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1. L. Pauling, H. A. Itano, S. J. Singer, and I. C. Wells. Sickle-cell Anemia, a Molecular Disease. Science 110, 543 (1949).

2. J. V. Neel, The Population Genetics of Two Inherited Blood Dyscrasias in Man. Cold Spring Harbor Symposia, Quant. Biol. 15, 141 (1951).

3. P. Brain. Sickle-Cell Anemia in Africa. Brit. Med. J. ii, 880 (1952).

4. A. C. Allison. Protection Afforded by Sickle-Cell Trait Against Subtertian Malarial Infection. Brit. Med. J. i, 290 (1954).

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The first step in solving a problem is to understand it. The discovery of the abnormal hemoglobins has provided us with a far deeper understanding of the hereditary hemolytic anemias than existed before. In the same way,

much may be done in increasing our understanding of phenylpyruvic oligophrenia. It may be found that there are many different allelomorphic genes that can contribute to the production of the disease; that is, that these genes, in homozygous state or double heterozygous state, may produce any one of a large number of somewhat different conditions that are now grouped together, and that some of the patients, who manufacture abnormal enzyme molecules that retain a certain amount of catalytic activity, may be susceptible to treatment. A test that would distinguish the carriers of phenylpyruvic oligophrenia would be useful; in particular, the siblings of a phenylpyruvic oligophrenic patient could learn whether or not he is a carrier of the gene, and whether or not he should avoid marrying another carrier.

A few months ago, when I gave the Ethel B. Ford Lecture[5], I mentioned that 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, and that it seems to me to be not unlikely that there are thousands of such diseases. I continued by saying that 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 - and this will require that a determination be made of the detailed arrangement of the thousands of atoms that make up one of the molecules of the enzyme - 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 in an artery of a new-born child who has been shown by the presence of phenylpyruvic acid in the urine

to have inherited phenylpyruvic oligophrenia; 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 of 2005 will turn out not to be a bold one, but rather a timid and unimaginative one.

These hereditary diseases involve the genes - abnormal genes, abnormal molecules that we can now safely identify as molecules of deoxyribosenucleic acid. Recent advances in knowledge about the structure of deoxyribosenucleic acid have provided the basis for confident speculation about the molecular nature of the processes of heredity. For fifteen years there has existed strong support for the belief that biological specificity in general involves a detailed complementariness in structure of interacting molecules,[6] and the proposal was made ten years ago[7] that the mechanism of self-duplication of the gene is a two-stage mechanism involving the use of a molecule A as the template for the synthesis of a complementary molecule A^{-1}, and then the use of A^{-1} as the template for the manufacture of a molecule complementary to it and identical with A. Watson and Crick[8] then made an extraordinarily attractive and stimulating proposal about the structure of deoxyribosenucleic acid. They showed that the x-ray diffraction pattern given by fibers of deoxyribosenucleic acid is compatible with a proposed structure involving two deoxyribosenucleic acid chains, coiled about one another to form a double helix. In each chain there are residues of one of the four nitrogen bases adenine, thymine, guanine, and cytosine at positions every 3.3 Å along the axis of the double helix. The structure is of such a nature that at each level the nitrogen bases of the two residues combine with one another through the formation of hydrogen bonds. The stable hydrogen-bonded structures that can be formed are only four in number: they involve having one

or another of the four pairs adenine-thymine, thymine-adenine, guanine-cytosine, or cytosine-guanine at each level. The nature of the hydrogen bonds formed by these pairs is shown in Figures 1 and 2 (these figures are from a paper by Pauling and Corey[9]; they differ from the proposal by Watson and Crick in showing three hydrogen bonds between guanine and cytosine, rather than only two). Accordingly the distribution of the four nitrogen bases along one polynucleotide chain is completely determined by that along the other: if adenine occurs at a given level in one chain, thymine must occur in the other, and so on. The molecular mechanism of inheritance proposed by Watson and Crick is accordingly that the double helix is untwisted, and each of the polynucleotide chains, A and A^{-1}, then serves as the template for the manufacture of its complement, A^{-1} and A, respectively. Although there are many details that need to be worked out, and some significant changes in this picture may well have to be made, there is no doubt, in my opinion, that Watson and Crick have made a contribution of great importance, and that we are now ready to attempt to formulate a completely detailed molecular mechanism of heredity, and to work out a thorough understanding of disease in terms of molecules. I am confident that, in particular, there will be rapid progress in the field of mental retardation and mental illness, during the coming decade.

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5. L. Pauling, The Future of Enzyme Research, Henry Ford Hospital Medical Bulletin, 4, 1 (1956).

6. L. Pauling, A Theory of the Structure and Process of Formation of Antibodies, J. Am. Chem. Soc. 62, 2643 (1940).

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

8. J. D. Watson and F. H. C. Crick, Molecular Structure of Nucleic Acids: A Structure for Deoxyribosenucleic Acid. Nature, 171, 737 (1953).

9. L. Pauling and R. B. Corey, Specific Hydrogen-Bond Formation Between Pyrimidines and Purines in Deoxyribosenucleic Acids, Linderstrom-Lang Festschrift.

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Legend for Figures

Fig. 1. Diagram showing complementariness in structure of thymine and adenine, which form two hydrogen bonds with one another.

Fig. 2. Diagram showing complementariness in structure of cytosine and guanine, forming three hydrogen bonds.