Genetics and dental health

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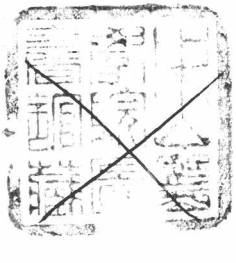

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Foreword

The publication of this book calls attention to a field of research endeavor that must surely claim greater emphasis in the years to come. It highlights the work and the thoughts of a number of pioneers whose efforts have now clarified the beginnings and fashioned a framework for the study of genetics in relation to dental problems as an emerging branch of scientific investigation.

The twenty-seven investigators who report in the following pages represent many professions and a variety of scientific disciplines in all parts of the world. Their common interest in the betterment of human health has drawn them together intellectually over many years through the regular media of scientific and professional communication. It was the privilege of the American Dental Association and the National Institute of Dental Research to bring so many of them together in reality, at the multidisciplinary Symposium in April, 1961.

The time appeared ripe to gather existing information on genetics related to oral health and disease, and to make it readily available to teachers and investigators. The Council on Dental Research recognized also that direct contact between scientists would promote the exchange and cross-fertilization of ideas that is so critically essential to scientific and philosophical advancement. The symposium accomplished significant progress along both of these lines.

The Council on Dental Research, in keeping with its responsibility to foster and improve research in areas of importance to dentistry, is pleased to have had a part in presenting the Symposium and this published record. The brilliant guidance and assistance of Dr. Carl J. Witkop, Jr., are deeply appreciated by the Council; his excellent editorial collation of the material will be appreciated by all who read this book.

A summary report of the Symposium appeared in *The Journal of the American Dental Association* in November, 1961. In that report was a statement, generated spontaneously in the enthusiasm of the Symposium and adopted by all the conferees, which urges more intensive study, teaching, and practical application of genetic knowledge. Already there are indications that this Symposium is only the first of a growing series of activities which seem certain to promote the spirit of that statement.

SHOLOM PEARLMAN, D.D.S:
Secretary, COUNCIL ON DENTAL RESEARCH

Preface

Recent advances in man's knowledge of the biochemical basis of heredity have had profound effect on every specialty of biological science. Dentistry has had a particular interest in genetics because many problems of facial growth and development, many defects of dentition and supporting structures, the dental aspects of evolution, and the effects of x-radiation relate to fundamental genetic processes.

Recognizing that new discoveries in genetics could offer better understanding and solutions of these dental problems, a committee established by the Council on Dental Research of the American Dental Association organized the first Symposium on Genetics Related to Dental Health, which was held in Bethesda, Maryland, April 4 to 6, 1961.

The Committee planned the conference with several objectives in mind:

1. To review the recent advances in genetics and to explore the ways in which these developments relate to areas of dental interest.
2. To bring together dental and genetic investigators who were working on problems of mutual interest for an exchange of information, methods, and ideas.
3. To stimulate interest and discussion among those conference members primarily concerned with dental education, as to the value of genetics as a discipline in the curriculum of dental schools and in the armamentarium of the practitioner.

This volume is compiled from the papers and discussions of the Symposium. It is the first book of this type devoted exclusively to dental genetic problems. While not intended to be a text, it does contain much factual and source material for the student of dentistry or genetics. The book does not include the excellent address, Genetic Effects of Radiation, presented to the Symposium participants at an informal session by Dr. William L. Russell of the Oak Ridge National Laboratory.

Special recognition must be given by the Committee to persons assisting in the Symposium: Dr. Francis A. Arnold, Jr., served as host; Drs. Thomas J. Hill, Seymour J. Kreshover, Robert M. Stephan, and William F. Swanson served as chairmen of the various sections of the Symposium; Dr. Harold Hillenbrand served as Toastmaster at the banquet; Dr. Sholom Pearlman and Mrs. Dorothy Vasilich of the Council on Dental Research handled the extensive correspondence and innumerable administrative

details of organizing the program and the publication; Mrs. Helen Miller helped to prepare and edit the manuscript; Dr. C. Willard Camalier, Mrs. Dorothy Horlander, Mrs. Hazel Dyson, Mr. Ronald Robinette, and Mr. Webster Leyshon helped with the local arrangements.

We hope that the interest initiated at this Symposium will spread throughout the dental profession and stimulate investigations of this fascinating aspect of human biology.

CARL J. WITKOP, JR., *Editor*

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The Biochemical Basis of Human Heredity

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In the past twenty years vast progress has been made in the understanding of human inheritance, especially in three directions which one could scarcely have dreamed possible in the case of an organism not amenable to experimental crosses. One of these directions lies in the study of population genetics; that is, the analysis of the frequencies maintained by varieties of genes in different populations and the nature of those evolutionary forces that modify those frequencies and alter the hereditary aspects of each population with time. A second direction lies in the exploration of the chemical nature of the hereditary material and its mutations, including the rates and causes of mutations and the damage thereby inflicted upon individuals and populations. At the same time, mutation affords all species their only hope of evolutionary novelty and successful adaptation to changes in the environment. The third direction is the exploration of the ways in which genes act within living cells and organisms, and especially the nature of the primary effect produced by each genetic change on the metabolism. This is in the strict sense the biochemistry of human heredity.

All life, in its biological aspects, is basically physical and chemical. We extend our comprehension of life processes as we discover the precise nature of the chemical changes that occur during growth, development, senescence, or illness, and the control over these processes that is exercised by the hereditary material transmitted from parents to offspring. The era of classical genetics (roughly 1900 to 1940) firmly established the chromosome theory of heredity. This theory posits: (1) that the hereditary units first discovered by Johann Gregor Mendel are present in the threadlike chromosomes in the nucleus of every cell; (2) that in the cells of sexually produced individuals, the sperm and ovum each contribute a complete set of

chromosomes, so that for every maternal chromosome there is a corresponding paternal chromosome and, with the exception of the sex chromosomes, for every maternal gene there is a corresponding paternal gene; and (3) that the pairs of genes belonging to a single pair of chromosomes are not transmitted from one generation to the next at random or independently, but in a linked manner which makes possible the mapping of the linear order of the genes within each chromosome.

The Chemical Nature of the Hereditary Material

To summarize briefly, the material of the chromosomes is chiefly deoxyribonucleic acid (DNA) and a strongly basic histone protein, along with lesser amounts of ribonucleic acid (RNA) and a different, less basic protein. This nucleoprotein transmits genetic information from one generation to the next in some form of chemical code. According to the prevailing theory it is composed fundamentally of a double helix of DNA, with the two coils polarized in opposite directions and firmly connected by thousands of hydrogen bonds between opposite nucleotides. The backbone of each helix is formed of alternating sugar (deoxyribose) and phosphate groups, with the various nucleotides projecting inward toward the central axis of the double helix. In most DNA there are only four types of nucleotides, those involving the two purines, adenine (A) and guanine (G), and the two pyrimidines, thymine (T) and cytosine (C). In human DNA a sizable portion of the cytosine may be replaced by methylcytosine (MC). In order to fit properly, according to the now generally accepted ideas of Watson and Crick,[1] nucleotides which are opposite and hydrogen-bonded to each other must always be A-T and G-C (or G-MC). In a single strand of the DNA the nucleotides can be arranged in any order and can occur in any proportions; but the order in one helix necessarily determines the particular complementary order in the other. In various kinds of DNA the ratio of the amount of adenine and thymine to the amount of guanine and cytosine is approximately 1.6:1; but in the common colon bacillus *Escherichia coli* it is 1:1. The histone protein of the nucleoprotein is probably coiled around the common axis of the double helix between alternate gyres of the DNA, and is probably weakly bonded to the latter. It remains uncertain how the RNA and the other protein are organized with respect to the double helix of DNA.

It used to be thought that only protein could provide all the variety of chemical structure necessary to supply a code of hereditary information. The new picture indicates that DNA, in its sequences of nucleotides, is also sufficiently complex; and today quite convincing evidence exists that DNA is the primary hereditary material, although that fact does not exclude protein from a coequal status, since the coded information might be transferred at times from one material to the other and back again. The most

critical evidence that DNA is the primary genetic material is that the hereditary types of certain bacteria (e.g., *Pneumococcus, Hemophilus*) can be permanently transformed by treatment with extremely pure DNA derived from related bacteria of a different hereditary type.[2] This evidence is reinforced by studies of bacterial viruses (bacteriophages), which inject their hereditary material into their host bacteria. The studies show that they inject *all* their DNA but only an insignificant part of their protein. Moreover, when the protein and nucleic acid of two varieties of tobacco mosaic virus (which contains RNA in place of DNA) are artificially separated and recombined—the protein of each one with the RNA of the other—the hereditary character of the resulting artificial virus is always that derived from the type which supplied the nucleic acid and never that of the type supplying the protein. Among higher organisms, it is notable that the wavelength of ultraviolet light that is most potent in making genes mutate is exactly the principal wavelength at which nucleic acid absorbs ultraviolet, whereas the wavelengths absorbed by protein are much less effective.

Among the major biochemical discoveries of our time have been those relating to the synthesis of the nucleic acids outside the living organism. Severo Ochoa[3] first isolated from a microorganism an enzyme that, when supplied with the four necessary nucleotide diphosphates in vitro, could synthesize an artificial RNA. Arthur Kornberg and his coworkers[4] shortly thereafter isolated a different enzyme from *E. coli* that, when supplied with the four nucleotide triphosphates, some adenosine triphosphate (ATP) to supply energy for the reaction, and a small amount of native DNA, can synthesize DNA which is indistinguishable in chemical nature from the DNA used as a model or primer. That is to say, if the DNA model is bacterial in origin, then bacterial DNA, to judge by its A-T/G-C ratio, is made. If the DNA model comes from calf thymus, then the DNA synthesized is like calf DNA in composition. Although no one has yet been able to introduce this artificial nucleic acid into a living cell and replace one gene with another, it is by no means unlikely that artificial genes may soon be made in this way for introduction into cells carrying defective genes.

The Chemical Nature of Mutation

The earlier studies of mutation were nearly all dependent upon spontaneous occurrences of mutation or upon the induction of mutations by means of ionizing radiations. The latter are extremely potent mutagenic agents, but they have a "shotgun effect," and tell us little of the chemical nature of the changes induced. During World War II more promising studies with chemical agents that produce mutation were initiated, and mustard gas, nitrogen mustard, and ethyl urethane were found to be highly effective. As time passed, and a variety of chemical agents were tested for mutagenic action, it became apparent that these agents differ in specific effects, some

of them calling forth certain mutations more frequently, and others eliciting other mutations more frequently. One might, then, hope ultimately to understand the mutation process in terms of the exact kinds of changes in the genetic material that would evoke certain particular mutations.

When it became clear that DNA is the primary genetic material, many investigators thought of using chemical agents that would act on DNA in some highly specific way. Thus, deoxyribonuclease, which breaks the phosphate-sugar backbone of the molecule, was tested and found to be highly mutagenic. Purine and pyrimidine analogues of adenine, guanine, thymine, and cytosine were tried, and some of them proved to be mutagenic. Caffeine, 5-bromouracil, and 2-aminopurine all act as mutagens; but while bromouracil and aminopurine appear to do so by substituting themselves for the regular bases during the replication of DNA, and so eventually leading to a replacement of some A-T pair by a G-C pair, or the reverse, it is apparent that caffeine cannot become actually incorporated into the DNA but must produce mutations more indirectly.

A strikingly specific effect is produced by nitrous acid, which attacks cytosine and adenine and deaminates them. It has been demonstrated that fruit flies, which mutate when formaldehyde is added to the medium on which they are raised, do so only if the medium contains adenine; so that, here again, the presence of a deaminated (formylated) purine base in the system brings about mutations. A new era in the study of the composition of the genes is beginning with these analyses of the mutation process that reveal the presence of A-T or G-C pairs of bases in some genes and not in others, or more in some and less in others.

The Control of Genes Over Specific Metabolic Steps

The chemical nature of DNA not only determines its capacity to mutate, and the nature of the mutations that arise, but also its capacity to replicate itself and transmit to future generations the chemical code that is the basis of heredity. It likewise determines the capacity of the DNA to establish and maintain the organization of the cell so that each of the numerous necessary enzymes is produced and performs its function, that energy is released from fuel substances and is utilized for work, and that the structural organization of the cell is built up and preserved.

Interestingly enough, our present insight into the way in which genes act, stemming largely from the work of George W. Beadle and Edward L. Tatum[5] on the inheritance of nutritional requirements in the pink bread mold *Neurospora,* took its inspiration from some almost forgotten studies of human hereditary anomalies conducted in the early years of this century. The British physician, A. E. Garrod, began in 1897 to investigate several cases of a rare and relatively harmless hereditary condition in which the urine, upon exposure to air, turns black—much to the consternation of

mothers who become alarmed at the staining of their babies' diapers. This condition was named alkaptonuria, and in 1902 became the subject of a classic discussion by Garrod of the hereditary nature of biochemical disorders. In 1908, in his Croonian lectures, Garrod [6] added to his list of what he felicitously called "inborn errors of metabolism" three other hereditary anomalies: albinism, cystinuria, and pentosuria. His most significant insight lay in the interpretation of the causes of these disorders. He suggested that in metabolism there are many sequences of biochemical steps: A → B → C → D, etc.; and that the hereditary biochemical deviations from normal in each case result from the blocking of a single step in such a sequence. The reason for such a block, Garrod supposed, lay in the failure of the body to produce the enzyme needed to control and mediate the particular step in question. In the case of a block between B and C in the sequence, the failure to form the product C, or an accumulation in excess of the substrate B from which C is formed, would bring about the pathologic symptoms.

Many years later, by applying their ingenious techniques to *Neurospora,* Beadle and Tatum demonstrated that Garrod's views of the nature of "inborn errors of metabolism" were indeed correct; and they ushered in a new era of the analysis of genetic effects on a biochemical level. Both genetics and biochemistry have been revolutionized. A prominent part in this advance has been played by the famous "one-gene–one-enzyme" or "one-gene–one metabolic step" hypothesis, which assumes that each gene controls the formation of a single protein, usually an enzyme, and consequently controls a single metabolic step, so that mutation of a particular gene blocks a particular biochemical step. Doubtless, there are instances where the gene may affect two proteins or where its mutation might affect more than one metabolic step; but clearly, most genes follow this law. The hypothesis has indeed been of enormous value in assisting the analysis of biochemical pathways, just as it has reoriented thinking within the field of genetics. In the past decade the methods developed in biochemical genetic studies of *Neurospora* and of bacteria have been modified and employed in the study of human hereditary variations. Thus, Garrod's vision of a fuller biochemical understanding of inborn errors of metabolism is being fulfilled.

Phenylketonuria

To illustrate the principles of gene action, one might consider our present knowledge of Garrod's classic case, alkaptonuria; but a somewhat better example is afforded by another disorder which is concerned with the metabolism of the same two amino acids, phenylalanine and tyrosine. This inborn error is known as phenylketonuria. It is readily detected because the urine of an affected person contains an abnormal excreted sub-

stance, phenylpyruvic acid; and when a 5 per cent ferric chloride solution is added to urine containing this keto acid, a blue-green color develops. In the urine of normal persons there is no detectable phenylpyruvic acid.

The blocked step in the metabolism of these affected persons is the conversion of phenylalanine into tyrosine by the simple addition of a hydroxyl group to the aromatic ring in the para position to the amino acid sidechain. Reasoning like Garrod or like a *Neurospora* geneticist, we might suppose that individuals whose metabolism is blocked at this step might suffer a serious, or even fatal, consequence because of a shortage of tyrosine, which is a most important substance in a variety of metabolic activities. Tyrosine is used not only in the synthesis of virtually all proteins, but also it is converted into adrenalin, the emergency hormone of the body; into the melanin pigments of the hair, skin, and eyes that protect us from excessive light; and into thyroxin, which regulates the level of the basal metabolism in the body. Tyrosine is also broken down in a series of steps—one of which is the very step blocked in alkaptonuria—into simpler compounds (fumaric acid and acetoacetic acid) which enter the respiratory citric acid cycle and are thus used as fuels. This pathway is the outlet, or spillway, for excess amounts of phenylalanine and tyrosine in the diet, and one should note that phenylalanine may be disposed of through this channel only when it is first converted into tyrosine. Thus tyrosine is in many ways a key metabolite; and yet it is clearly not any lack of tyrosine that leads to the abnormal consequences in this disorder. Tyrosine is obtainable, in any normal diet, in quite sufficient quantities from the digestion of meat, milk, eggs, and other protein foods. The only evidence of even the slightest tyrosine deficiency in these persons is a degree of bleaching of the hair and complexion owing to an insufficient production of melanin; but this very minor effect arises, not because of insufficient tyrosine, but rather because the excessive amount of phenylalanine accumulated (caused by the blocked step) has itself an inhibitory effect on one of the enzymes involved in the production of melanin pigments.

In short, the high level of phenylalanine in the blood plasma, some twenty to sixty times above normal, is the key biochemical effect arising from the blocking of the conversion of phenylalanine into tyrosine. As a consequence of this primary effect, a number of secondary consequences arise. One of these is the production and excretion of phenylpyruvic acid, already mentioned; while from phenylpyruvic acid quite a number of other unusual products are formed and excreted through the kidneys. The most important effect of the disorder, from the standpoint of human welfare, is the failure of these persons to develop a normal mentality. Their intelligence, as measured by IQ tests, generally remains at a level of idiocy or imbecility, although about 2 per cent of cases have an IQ above 60. Occasionally even an IQ of 70, in the moronic grade, is surpassed, and as the ferric chloride test is more generally applied to all newborn infants,

it may well turn out that a larger fraction of the phenylketonurics have fairly normal intelligence than is now supposed to be the case. As it stands at present, between 0.5 per cent and 1 per cent of the inmates of our mental institutions are made up of these unfortunates, although, in the general population of the United States, the frequency of the condition is little above 2 per 100,000 persons. The wide range in mental ability of the phenylketonurics is most significant. Evidently the metabolic error does not extinguish the wide range of mental ability arising among different individuals because of nature and nurture, that is, because of their environmental circumstances and, especially, their other genes. The same may be said of the increased blondness of these individuals and likewise of their generally smaller head size. Great variations still exist in these characteristics, and in general the distributions overlap extensively with the range of variation in the normal nonphenylketonuric population. One may suppose that it is only when other genes for blondness add to the effect of the phenylalanine blockage that striking blondness results; and again, that it is only when other genes provide a rather low intellectual capacity that the phenylalanine blockage depresses the IQ to the level of lowest idiocy.

On the other hand, the distributions of phenylalanine blood plasma concentration in the general population and in the phenylketonurics are quite distinct, and do not come even close to overlapping. Clearly, as we probe deeper into the nature of the effect of a genetic difference and arrive finally at the immediate biochemical consequence of the mutation, usually we find that the trait becomes distinct from other conditions—becomes, in fact, unique. It is when we examine the secondary and even more remote consequences of a genetic difference that we find other genes and a variety of environmental conditions participating in the formation of the characteristics, and the distinctness of effect of the original genetic difference becomes obscured. Nevertheless, as the consideration of phenylketonuria so clearly shows, a bimodal, or even a slightly bimodal, distribution of a character may reflect a difference of a single gene. Of course we should not say that the normal allele of the gene for phenylketonuria alone determines intelligence, nor that the gene for phenylketonuria alone produces idiocy or imbecility; but the latter shifts the normal distribution of intelligence so far down the scale that only those who, in the absence of the gene, might have been true geniuses will remain capable of the learning capacity of a moron. This interpretation is supported by the fact that among the phenylketonurics themselves there is no correlation between the level of the amino acid in their blood plasma, on the one hand, and their level of intelligence, on the other. Given the big shift down scale which is produced by the metabolic block, other factors determine the variation within the group; and the same may be said for each of the other effects already considered, the complexion, head size, and the like.

Even the accumulation of phenylalanine in the blood is a secondary

and not the true primary effect of the alteration of the gene in phenylketonuria. It has been demonstrated that, one step farther back, the enzyme which converts phenylalanine into tyrosine and is normally present in the liver is absent, or at least is inactive. Once the biochemical nature of a hereditary metabolic error is known, remedial measures may be conceived—for it is clearly no longer true to think of hereditary disease as being different from infectious diseases in being beyond hope of cure or alleviation. Look at what has been done for diabetes! In the case of phenylketonuria it is, of course, not feasible to introduce the missing enzyme into the liver cells of the defective children; for if taken by mouth, the enzyme molecules, like any protein, are quickly digested; and if injected into the blood stream, they may produce dangerous reactions, since the enzyme, unlike insulin, has not been purified and may not be low in antigenicity. However, phenylketonuric children can be supplied a diet low in phenylalanine. Some is, of course, necessary to maintain life and growth, and up to 1 gm of phenylalanine can be administered daily without a rise in the plasma concentration. On a protein-free diet supplemented with all the other amino acids in normal amounts and with phenylalanine in the minimal amount required for health, a remarkable improvement in mental development is possible in a phenylketonuric child if the treatment is started early enough. Actually, for proper control and alleviation of the condition, the diet should be started at birth, since the casein of milk contains considerable amounts of phenylalanine. Improvement cannot be brought about after the condition has persisted for a number of years.

Phenylketonuria is a simple recessive trait, appearing among the progeny of two nonaffected parents who are both carriers of the gene. The probability of a child's being affected, in such a progeny, is one-fourth. As in the case of nearly all rare hereditary disorders that are recessive traits, the incidence of consanguinity among the parents is greater than in the general population as they both are more likely to be carriers. In the case of phenylketonuria, the parents show none of the ordinary symptoms of the condition. Their intelligence varies within the range of the general population; they do not excrete phenylpyruvic acid; and they do not show unusual blondness. Yet a critical test, imposing a stress on the enzymatic system which converts phenylalanine into tyrosine, proves them to be distinguishable from noncarriers of the gene.[7] When subjected to a high dose of phenylalanine, they are unable to utilize or excrete it as rapidly as noncarriers of the gene. Moreover, when a recently improved, highly sensitive method of determining the phenylalanine concentration of the blood plasma is used,[8] the average of the carriers is significantly different from that of noncarriers, although there is some overlapping of the two distributions. In other words, the so-called recessive gene is not really recessive when a critical biochemical test is applied to the heterozygous individual. Perhaps such persons have only half the normal amount of the enzyme, phenylalanine hydroxylase, and this is quite

sufficient to handle ordinary amounts of phenylalanine. It appears that the body normally operates with a considerable safety factor, or genetic reserve, since it usually possesses two genes of every kind. Recessiveness results because only one is really needed to satisfy the metabolic demands.

It is almost unnecessary to consider further examples of human biochemical genetics, so fully are the general principles exemplified in the case of phenylketonuria. We see here a single gene difference, recessive in inheritance, that blocks a single metabolic step by reason of the absence of the essential enzyme. The otherwise lethal effect of the deficiency of the essential product of the step, tyrosine, is obviated by the presence of tyrosine in the normal protein constituents of the diet; but the accumulation of phenylalanine, the substrate of the reaction, is very marked. A variety of unusual by-products of the dammed-up phenylalanine results, and these are excreted by the kidneys. A toxic effect of phenylalanine or some by-product is exerted on the development of the nervous system and leads to a low grade of mentality. Other secondary and more remote consequences are observable. In determining the level of intelligence, and in the other secondary effects, the remainder of the genotype and the variations in the environment play significant parts, and the affected and normal populations as a consequence overlap considerably, even though the initial observable effect, the high level of phenylalanine in the blood, is quite distinct. The hereditary defect, once its nature is known, becomes subject to amelioration, although one must remember that the defective gene is not modified by the treatment and will remain to be passed to future generations if the restoration to normal life enables the affected person to mate and reproduce. The consequence to the population is not good, since the frequency of defective genes in the population is increased by such measures and the number of affected individuals born will also gradually increase. Finally, it is most significant that the heterozygous parents can be detected, in spite of their lack of general symptoms, by delicate tests of their phenylalanine tolerance and phenylalanine plasma levels. By sufficient general testing of the population, particularly whenever cousin marriages are contemplated, the production of socially undesirable progenies might be avoided.

Galactosemia and Nonhemolytic Familial Jaundice

A few additional points may be brought out by other examples. Galactosemia is a recessive hereditary inability to utilize the sugar galactose. Herman Kalckar and his associates[9] first worked out the steps normally involved in the utilization of galactose, which, along with glucose, is derived from the digestion of the milk sugar, lactose. Interestingly, their study began not with human beings, but with the biochemistry of the common colon bacillus, *Escherichia coli*. Mutants of the colon bacillus which were