BIOCHEMICAL GENETICS

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I. INTRODUCTION

Biochemical genetics may be defined as that branch of biology which seeks to define hereditary units in terms of the chemistry of their structures and of their functions. Mendel most probably wondered about the physical nature

of the unit factors he postulated and about the manner of their action in producing different types of pea plants—for example, those with purple flowers and those with white ones. Certainly, since the rediscovery of his paper in 1900 many others have thought about questions concerning the nature of genes, the equivalents in modern terminology of Mendel's factors.

Biochemical genetics is a field cultivated more by the biologist than by the biochemist. The biologist has been insistently pushed in this direction by advances in genetics, while the biochemist has so far found nothing inherent in his subject that tends so to urge him. History might well have been otherwise, for, as this review attempts to make clear, genes are as much a part of biochemistry as chemistry is of inheritance, development, and function.

The present review attempts to bring together certain facts of genetics and to indicate how these bear on hypotheses concerning gene structure and gene action. For the most part topics have been restricted to those in which facts and interpretations can be expressed in terms of known compounds or reactions about which at least something is understood from a chemical standpoint. Thus, much material often assigned to "physiological genetics" or to "developmental genetics" is omitted. For treatments of material from these viewpoints the reader is referred to Wright (347), Waddington (327, 328), and Goldschmidt (110). For more extensive treatments of "classical genetics," i.e., the mechanics of gene transmission, reference is made to standard textbooks on the subject (66, 268, 276, 308, 327).

II. THE CONCEPT OF THE GENE

The classical geneticist deals with the gene from the standpoint of its behavior in transmission from parent to offspring. In this sense it may be defined as the irreducible unit of inheritance. For the elaboration of a purely formal theory of the type proposed by Mendel, nothing need be assumed as to the physical nature of the gene or its mode of action other than that it somehow causes differentiation of alternative characters in the organism. For example, there are two categories of persons with respect to ability to taste phenylthiourea. To some this is a disagreeably bitter substance not unlike quinine, while to others it is quite tasteless. The mechanical inheritance of this trait can be adequately accounted for by assuming two forms of a factor (gene), one of which conditions the ability of the individual to taste phenylthiourea. The individual receives one or the other of the forms of this gene (alleles) from each of his parents. With respect to individuals there are therefore three combinations of alleles possible, *viz.*, tasters who receive a taster allele from each parent, tasters who receive a taster allele from only one parent, and non-tasters who receive a non-taster allele from each parent. The ability of the second type of person to taste is ascribed to the dominance of the taster form of the gene over its allele.

Actually, of course, it has been known with certainty for some thirty years that genes are carried in chromosomes. Again, from a purely formal point of view it is not a necessity that chromosomes be visible; they could have been predicted from the observations of the inheritance of traits such as taste reac-

tion. This is not to deny that their identification as carriers of genes has been a most powerful factor in advancing the science of genetics.

A view widely held at the present time, therefore, is that genes are units of inheritance, carried in chromosomes which correspond to the linkage groups of the geneticist. The identification of the chromosomes as the carriers of genes is made on the basis of the complete parallelism between the behavior of these bodies as observed under the microscope and the behavior of the genes as inferred from inheritance data. The genes are arranged in a linear order in the chromosomes, and their positions within these linear groups can be determined genetically by using probabilities of recombinations of linked genes as measures of distances. Recombinations of linked genes result from exchange of corresponding segments of homologous chromosomes during the process of chromosome reduction (meiosis). Gene positions (loci) can be independently determined by correlating visible chromosome aberrations with corresponding aberrations in inheritance, e.g., losses of visible segments of chromosomes lead to losses of the genes carried in them.

Genetically it is possible to identify genes only under special circumstances. In the first place, it is not possible to recognize a given gene unless it exists in at least two forms. This can be illustrated by the example of taste reaction to phenylthiourea. If each individual of the human species received a taster allele of this gene from each parent and was therefore homozygous for this form of the gene, there would be no way of inferring that this particular gene existed. Different forms of a gene arise by mutation. As we shall see, gene mutations involve changes of an unknown nature. They may occur spontaneously or their frequency may be artificially increased by treatment with high-energy radiation, such as ultraviolet light, x-rays, and neutrons. A special type of change by which genes can be identified is complete loss.

A second requirement in experimentally identifying a given gene is that its mutation to a new allele, or its loss, results in a detectable change in the organism. Failure to detect a gene-controlled modification may be due to its being quantitatively too small for detection, to there being no suitable qualitative technique for detection of the change, or simply to failure to look for the modification. All of these are experimental limitations. They can be illustrated by an example in nitrogen metabolism. Most mammals, including most races of dogs, excrete purine nitrogen in the form of allantoin. In the Dalmatian coach hound, however, this nitrogen is largely in the form of uric acid. In this respect the Dalmatian differs in one gene from other dogs (322). Knowledge of the existence of this gene is dependent on the detection of the difference in urinary nitrogen. There is no necessary difference in gross appearance between dogs that excrete allantoin and those that excrete uric acid.

In contrast to the possibility that substitution of a given gene has a final effect too small for detection, there is a real possibility that, from the standpoint of the investigator, the effect may be too great. For example, if a given single gene substitution were to prevent cell division, the result would be lethal to any multicellular organism. In certain organisms such characters could be

worked with, and even in man it would be possible to infer the existence of a gene concerned with such a vital phase of early development if sufficient genetic data were available.

The genetic definition of a gene implies sexual reproduction. It is only through segregation and recombination of genes during meiosis and fusion of gametes that the gene exhibits its unitary property. In bacteria, for example, in which cell reproduction is vegetative, there are presumably units functionally homologous with the genes of higher organisms (119, 250a), but there is no means by which these can be identified by the techniques of classical genetics.

It is perhaps worth pointing out that because mutation rates for specific genes are usually low, even following drastic treatment, and because most changes that do occur probably remain undetected experimentally, the majority of the genes of a given organism remain unknown. Even in the vinegar fly *Drosophila melanogaster*, the classical organism of genetics, there are probably at least twenty times as many genes as the few hundred known to geneticists. The detection of such a gene as that for taste reaction to phenylthiourea would not be simple in a vinegar fly.

The biochemical geneticist is concerned with the gene from the standpoint of its chemical structure and its function. He works on the assumption that it is possible to define the gene in chemical terms. Because of limitations in knowledge, such a definition is at present in many respects less satisfactory than that based solely on inheritance. But it can be hoped that the future will see these limitations reduced in number.

It has been seriously argued by Goldschmidt (110) that from a functional standpoint the gene is an artifact, but the evidence for this point of view is not convincing to most biologists. The contrary approach has at least demonstrated its value in stimulating progress. Accordingly, until it is proved otherwise, we are justified in assuming that, however the approach is made, in the end the gene will prove to be an identifiable unit.

If the biochemical geneticist should attain his goal of defining the gene in terms of one or more unique functional properties, many of the experimental limitations discussed above would be eliminated and the scope of the genetic method correspondingly broadened.

III. HYPOTHESIS OF GENE STRUCTURE AND GENE ACTION

As a framework on which to arrange conveniently the varied observations and inferences bearing on the nature of genes and their action it is desirable to have set down a definite summary hypothesis. The presentation of such a hypothesis is undertaken with the realization that alternatives are not only possible but in at least certain respects equally plausible. References are given in connection with later detailed discussions of individual points.

Gene structure—The gene is made up of protein or nucleoprotein. It may correspond to a single giant molecule, or it may be a discrete unit of higher order made up of a group of protein or nucleoprotein molecules, with or without the addition of other substances.

Self-duplication—In order to exist as such, genes obviously must be capable of inducing the formation of exact copies of themselves. The way in which such self-duplication occurs is not known but is presumed to involve some type of model-copy mechanism.

Heterocatalysis—In addition to catalyzing formation of more units like themselves, genes in general have heterocatalytic properties, that is, they catalyze the formation of other substances. The hetero- and auto-catalytic functions are probably essentially similar and consist of imposing specific configurations on protein or other molecules in the final step in their synthesis.

Relation to specific chemical reactions—In determining the specific chemical and perhaps physical configurations of protein molecules, genes directly determine enzyme specificities and thereby control in a primary way enzymatic syntheses and other chemical reactions in the organism.

Gene specificity—Each nucleus of those organisms sufficiently advanced in the evolutionary scale to have nuclei contains many thousands of genes. In diploid nuclei these exist in pairs and in polyploids in groups of higher order. Each of these thousands of gene types has, in general, a unique specificity. This means that a given enzyme will usually have its final specificity set by one and only one gene. The same is true of other unique proteins, for example, those functioning as antigens.

Gene mutation—Through the absorption of energy, which may occur in a number of ways, genes may undergo mutation. If such a mutational change abolishes the autocatalytic property of the gene, the gene is irreversibly lost. On the other hand, if it loses only its heterocatalytic power it remains a gene, but so far as its effect on the organism of which it is a part is concerned it becomes inactive (an "amorph" in the terminology of Muller (327, 347)). Such inactive genes in homozygous forms are of course likely to be deleterious if not actually lethal to the organism. Other types of gene mutations presumed to be possible are those in which the heterocatalytic property is impaired but not destroyed (hypomorphs), those in which the effectiveness of heterocatalysis is increased (hypermorphs), and finally those in which there is a change in one step from one heterocatalytic specificity to another (neomorphs).

IV. CHARACTERS CONTROLLED BY GENES

Almost every aspect of the organism has been shown to be influenced by genetic constitution in one way or another. To indicate the general nature of these relations a series of examples is presented here. In some of these it is not possible at present to see how any simple interpretation can be made in terms of gene action. Nevertheless, they may suggest directions in which future advances can be expected. The sequence of presentation is roughly in the order of decreasing apparent complexity of the gene–character interrelation.

A. Psychological characters

In a number of instances specific behavior patterns are known to be influenced by genes. In man, in whom opportunities are greatest for detecting variations

of this kind, the direct methods of classical genetics cannot ordinarily be used. It is nevertheless possible to gain some information. The so-called twin method is a most useful one in this respect. Identical twins are identical genetically because they develop from a single fertilized egg. Fraternal twins, on the other hand, develop from independently fertilized but simultaneously developing eggs. Intrapair comparisons have been made in both types, with the members of the pairs reared both together and separately (222). Such studies show that differences in behavior can be correlated with differences in genetic constitution. As one might expect, there is no clue as to how such subtle differences are related developmentally to the particular genes that determine them. To illustrate the difficulty of interpreting gene action in such cases Wright (347) has used the example of the web-patterns of spiders. Here interspecific variations are presumably genetically determined. But the manner in which one form of a gene determines that a spider shall construct a web of one pattern while another form results in the construction of a characteristically different web must indeed involve a tortuous chain of events.

Several characteristic abnormalities of the nervous system of man have been referred to single gene modifications. Specific types of feeblemindedness are almost certainly the result of single gene changes. In one instance it is known that idiocy or imbecility is associated with inability of the individual to oxidize phenylpyruvic acid to its para-hydroxy analogue (98). Further details regarding this situation will be considered later; it suffices here to point out that it is through studies of just such instances as this that the way may be prepared for significant advances in our knowledge of the chemical basis of the functioning of the central nervous system. It is an illustration of how advances come in unexpected ways, and reinforces confidence in the hypothesis stating that, no matter how complex the change may appear to be when one gene allele is substituted for another, the primary modification will ultimately be found to lie in a single chemical reaction.

Another example in which an unpredicted correlation between genetic constitution and response of the central nervous system has been found involved the disease epilepsy. This condition has long been suspected of being hereditary, but its genetic basis appeared not to be simple. Recently Lennox, Gibbs, and Gibbs (174) have found that the brain-wave pattern, revealed by recording fluctuations in potential set up between two electrodes in contact with the head, is characteristic among those predisposed to epileptic seizures as compared with those not so predisposed. The disrhythmia characteristic of epileptics is inherited as a simple Mendelian dominant character. Fortunately, only about one in twenty persons with this brain-wave pattern ever develops epilepsy. Here again we appear to be a long way from a chemical interpretation, but one can nevertheless say that a first step has been taken.

Taste reaction of individuals to phenylthiourea has already been mentioned. Red–green color blindness is a classical example of a sex-linked recessive trait in man. Since the Y chromosome carries no allele of this gene, all males who carry the mutant allele of this color vision gene in their X chromosomes show

the trait. The female, having two X chromosomes, requires a mutant allele from each parent to show the character. As expected on the basis of mating occurring at random with respect to color blindness, the frequency of the defect in women (about 0.6 per cent) is roughly equal to the square of its frequency in males (about 8.0 per cent). Many other instances of inherited differences in sensory perception probably remain to be discovered. Each individual of a species is likely to be unique in response to the external world because of a unique combination of genes received from his two parents.

B. Genes affecting chromosome behavior

While the physical mechanisms by which genes are transmitted from one generation to the next are remarkably stable in the evolutionary sense, several variations are known. Bacteria have no sexual reproduction as far as we know, and the partitioning of the chromatin at cell division appears to differ significantly from that of higher plants or animals. If gene change is the basis of evolutionary divergence, it follows that these and lesser variations in the mechanics of gene transmission are themselves subject to gene control (65). In fact, there is direct genetic evidence that this is the case. There are now recorded many instances in which abnormalities of one kind or another in cell division are ascribable to specific substitutions of one gene allele for another (65). For example, genes for asynapsis (failure of chromosomes to pair or remain paired during the first meiotic division and leading to irregular chromosome distribution) are known in maize, wheat, rice, barley, cotton, Jimson weed, peas, onion, blue-eyed grass, and the vinegar fly (22, 50, 271, 272). Genically determined failure of cytoplasmic division leading to excessive multiplication of the chromosomes in certain cells is known in corn, wheat, and barley (13, 273). The converse, division of cytoplasm without chromosome division, is also known (12). Possession of divergent spindles at the first meiotic division is a simple recessive trait in maize (50). General "stickiness" of chromosomes leading to breakage, rearrangement, and other aberrations is known to be a recessive character in maize (14).

Cell division is without doubt a complicated process. Yet, if one assumes that each of the gene-controlled deviations mentioned above results from blocking or modification of a single chemical reaction, there would seem to be some hope of finding leads that would bring one closer to a physicochemical interpretation.

C. Gene stability as a genetic trait

Genes vary in their stabilities. So far as can be determined, some of those in the vinegar fly (*Drosophila*) have mutated only once in its thirty-five years history as a laboratory animal (some five hundred generations with hundreds of thousands of individuals under observation in each generation). Others probably exist that have not mutated in a detectable manner during this time. On the other hand, there are many so-called unstable genes in which mutations occur hundreds of times in the development of a single individual (71). That

such differences in mutation rate can be subject to genetic control is shown most clearly by Rhoades (242, 243a). In maize the development of anthocyanin pigment in the aleurone layer (outer layer of the endosperm of the kernel) and in leaves, stems, and other parts of the plant is dependent on a series of non-allelic genes. One of these is the *A*,*a* pair of alleles. If favorable alleles of all other genes are present, the difference between color and no color is determined by whether a plant (or part of it) has in its cells one or two *A* alleles (i.e., *AA* or *Aa*) or only *a*. The *a* allele is usually very stable. However, in the presence of a dominant allele of the *dotted* gene, which is in an entirely different chromosome pair from the *Aa* alleles, allele *a* undergoes frequent mutation to *A* or other dominant alleles. This is detected by observing patches of colored aleurone or similar colored areas on other parts of the plant. This genic control of specific mutation is significant in several respects. For one thing, it indicates clearly that the *a* allele does not represent merely loss of the *A* gene. Rather, it appears to be a true amorph; it retains the property of autocatalysis but has lost its heterocatalytic function. It is interesting to note that in this instance we know that the gene whose stability is controlled is concerned with some reaction in anthocyanin synthesis but that we have no idea by what means the controlling gene is accomplishing its end result.

D. Morphogenesis

Any treatment beyond mere description of the development of form in organisms cannot be divorced from consideration of the development of function; the first is the physical manifestation of the second. The development of the morphological patterns of higher plants and animals is clearly subject to genetic control, but in contemplating the manner in which this control is brought about we immediately encounter the problem of how cells, tissues, and organs can be differentiated when supposedly all the component cells, being derived by mitotic divisions from the fertilized egg, have identical sets of genes. A blood cell and a nerve cell, for example, differ markedly in both form and function. What is the evidence that they are identical genetically? Why have we rejected views such as those of Roux and of Weismann (339), who held that somatic cell divisions involved *qualitative* divisions of the nuclei by which "determinants" differentially assorted to various parts of the body and gave rise to the many types of cell, tissue, and organ differentiation? Direct proof of the genetic identity of different types of somatic cells is difficult to obtain. We observe that the mitotic divisions of the nuclei accompanying cell division appear to distribute daughter chromosomes equally to daughter cells, but this in no way denies the possibility that genes mutate in a regular fashion and thus account for the process of differentiation. Direct proof would require that differentiated cells be dedifferentiated and shown to be capable of reproducing the whole organism. In higher plants and in some animals this can be approximated. For example, a segment of stem of a grape plant can be rooted, whereupon it gives rise to a new grape plant complete in every respect. But the cutting is known to contain many cells that have remained embryonic. A convincing demon-

stration requires the equivalent of removing the nucleus from a highly specialized cell such as one of nervous tissue, transplanting it to an enucleated egg, and from this reproducing a complete organism. Aside from demonstrating that one descendent nucleus after four nuclear divisions in the fertilized egg (giving sixteen nuclei) can, in the presence of the proper type of cytoplasm, replace the original egg nucleus perfectly in the salamander (281), no such crucial test has been made with higher organisms.

Fortunately there are two reasonably direct ways of showing that differentiation does not involve an irreversible genetic change. One is based on the behavior in development of the protista (single-celled plants and animals). Here, as for example in the ciliate protozoa or certain algae such as *Acetabularia*, described below, we have cellular differentiation carried as far or even further than in higher organisms and with complete reversibility. Certain protozoa with their elaborate system of organelles undergo complete dedifferentiation and redifferentiation at certain times in the life cycle, e.g., during encystment and excystment. Since there is clearly no irreversible genetic change in the process here, there is no need to assume one in the comparable differentiation of individual vegetative cells of the structurally more elaborate metazoa and metaphyta.

A second argument is based on the fact that in the eggs of many metazoa, regions of the cytoplasm are known to be irreversibly determined as to their subsequent fate *before* the division of the egg nucleus (339). It is clear from this observation of experimental embryology that the immediate cause of differentiation lies in the localization of "organ-forming" substances in the cytoplasm and that there is no need to postulate nuclear differences as a basis of ontogenetic divergence of structure and function.

We are therefore returned to the original paradox. Cells of identical genotypes travel many divergent paths in the process of differentiation of the individual organism. Enzyme differences are certainly involved, for it is well known that tissue types differ widely in content of these organic catalysts. This is true also for such substances as hormones, vitamins, and antigens. The agglutinogens that differentiate certain blood types in man, for instance, appear to be confined to the cells of the blood (301). The answer is that pattern differences are evidently set up early in development by apparently trivial circumstances (339). These initially minor differences undergo progressive amplification as development proceeds, leading to what eventually appears to be a most complex integration of form and function. As a specific example of how such a process is thought to work, we can cite the case of the seaweed *Fucus*, as studied by Whitaker (333) and others. The fertilized egg is apparently initially spherically symmetrical. Almost any conceivable asymmetry of the environment upsets this egg symmetry. If it lies on the bottom of a vessel containing the sea water in which it develops, a gradient is set up from top to bottom by differential ease of diffusion of oxygen to the cell and carbon dioxide away from it. In some manner not completely understood but possibly involving a gradient of amount or activity of growth hormone (indole-3-acetic

acid) in the egg, a rhizoid initial (root-like protuberance) is formed on the bottom of the egg. Cell division occurs with the division wall established at right angles to the vertical axis. The initial gradient can be established along any diameter of the egg. Subsequently the axial gradient becomes more and more elaborate (see Child (46) for comprehensive treatment of the rôle of gradients in development and differentiation). The initial minor environmental difference between the upper and lower parts of the egg sets off an orderly train of events that lead eventually to the fully differentiated seaweed. A most significant property of the *Fucus* egg as shown in the work of Whitaker and others is that the initial determination of polarity in the egg can be brought about by different environmental agents, among them: mechanical elongation, stratification of egg contents by centrifugation, temperature gradients across the egg, hydrogen-ion differentials, an electrical potential across the egg, and unilateral differences in illumination with either visible or ultraviolet light.

Although it may appear to be exceedingly difficult to determine in detail how genes exert their influence on a process as complex as differentiation, there are a number of cases in which at least a beginning can be made. One of these is a type of dwarf in the mouse studied by Smith and MacDowell and others (122, 274). This character is differentiated from normal by a single gene. The dwarf allele is recessive. Mice homozygous for this allele attain only about one-sixth the weight of their normal sibs, have defective thyroids, and are entirely incapable of reproduction. Histological examination shows that their pituitary glands are defective in being deficient in a particular type of cell (eosinophiles). If pituitary glands from normal mice are transplanted to dwarfs at the right stage of development, the genetic dwarfs develop into mice that are essentially normal. Their thyroids become normal, they attain an essentially normal size, and the males become fertile. The only defect that remains appears to be that of the anterior lobe of the pituitary. Here, then, the normal allele of the gene concerned evidently has something to do with the production of one or more of the growth hormones normally elaborated by the anterior lobe of the pituitary gland, and known to influence developmental processes other than growth.

Somewhat similar situations are known in the case of dwarfness in maize (325a), in biennial *versus* annual growth habit in certain plants (206), and in a number of other organisms (110, 221, 328).

Genes evidently determine the final potentialities of differentiation in a given organism. The initiation of the orderly process may be through the environment, but whether or not a given reaction in the time–space sequence can take place is gene controlled. If a given gene is present in a cell, the presence of an enzyme with a corresponding specificity is dependent on the antecedent steps in the developmental sequence. If these are right, it will be present, otherwise not. But if the gene is absent or defective, the enzyme cannot be formed regardless of what has gone before. Wright (348) has ably summarized this genetic point of view regarding developmental processes, but recognizes that its application as a whole in individual instances is not simple. What is needed

is a resolution of the problem into simpler components. This of course is constantly being done—at the morphological level by students of organizer phenomena, at a physiological level by those responsible for the development of gradient theories, and finally at a chemical level by the biochemist. The genetic approach has been much neglected. The possibilities of systematically studying changes brought about in morphogenetic processes by substitutions of single genes are virtually unlimited. If each change is really referable to a change in a single primary reaction, then the method should be a most powerful one.

E. Tyrosine–phenylalanine metabolism in man

During the present century several inherited errors having to do with the metabolism of the amino acids tyrosine and phenylalanine have been described in man. One of these in particular is a classic of biochemical genetics, because it represents the first instance in which a particular gene was related to a specific and known biochemical reaction. It is the disease known as alcaptonuria. One characteristic symptom of this metabolic deviation, blackening of the urine on exposure to air, was recorded three hundred sixty years ago (101). Eighty-seven years ago Bodecker isolated the substance responsible for this discoloration of the urine and found it to be 2,5-dihydroxyphenylacetic acid, also known under the names alcapton and homogentisic acid (101). Shortly after the rediscovery of Mendel's paper in 1900, Bateson and Punnett pointed out that alcaptonuria behaved in inheritance like a simple Mendelian recessive trait (10). In the first edition of *Inborn Errors of Metabolism*, published in 1909, Garrod (101) summarized the accumulated information as to the nature and inheritance of alcaptonuria. Five years later Gross (101) found that the blood serum of normal individuals contains an enzyme capable of catalyzing the breakdown of homogentisic acid. This enzyme is not found in the sera of alcaptonurics. Thus, thirty-one years ago, it was clearly established that a single gene substitution results in the absence or inactivity of a specific enzyme and that this in turn leads to the failure of a particular biochemical reaction. It is interesting that no clearer example exists today.

From a historical standpoint it is a curious fact that until recent years alcaptonuria has played almost no part in the development of theories of gene action. Garrod's book (referred to above) and its second edition, published in 1923 (101), treated alcaptonuria in detail from both the biochemical and the genetic points of view. This book, which also contains accounts of other inherited metabolic abnormalities in man, should be credited as representing the beginning of biochemical genetics, but unfortunately, until its significance was pointed out recently, particularly by Haldane, it has remained practically unknown to geneticists. The biochemists, it should be said, were less guilty of neglect, but they apparently were not prepared to appreciate fully the genetic implications.

Because the studies on alcaptonuria, many of which were carried out by Garrod himself, are of interest both historically and currently in illustrating methods of biochemical genetics, they are well worth consideration in some detail. (See Garrod (101) for references and additional details.) Normally, 2,5-

dihydroxyphenylacetic acid is broken down, probably by way of acetoacetic acid, to carbon dioxide and water. In alcaptonurics this breakdown is blocked. It would therefore be expected that normal precursors of homogentisic acid, or compounds convertible to such precursors by reactions carried out in the organism, would lead to increased excretion of this intermediate in the breakdown process, but would be completely metabolized by normal individuals. Following this reasoning a series of compounds has been fed to alcaptonurics and to normal controls. As expected, homogentisic acid ingested by alcaptonurics is excreted, but is broken down by normal persons. Both phenylalanine and tyrosine lead to increased homogentisic acid in the urine of alcaptonurics, but not in normal individuals, and are therefore judged to be initial precursors. *p*-Hydroxyphenylpyruvic acid is converted to homogentisic acid, while its lactic acid analogue is not. Because *p*-hydroxyphenylacetic acid is not converted to homogentisic acid, whereas 2,5-dihydroxyphenylpyruvic acid is, it is thought that the oxidation of the aromatic carbon atoms must precede that of the side chain. Gentisic acid (2,5-dihydroxybenzoic acid) is excreted by sufferers from alcaptonuria but is metabolized by normal persons. Benzoic acid oxidized at other positions in the ring is metabolized by both normals and alcaptonurics. It thus appears that the specific disability induced by the gene substitution is that of failure to disrupt the ring structure in 2,5-dihydroxyphenyl compounds.

A second inherited abnormality of phenylalanine–tyrosine metabolism in man is responsible for the condition known as albinism. Here melanin formation is greatly reduced. Again, our knowledge of this trait was brought together by Garrod in 1923 (101). In at least most instances albinism behaves as a simple Mendelian recessive character. Unfortunately, the chemical transformations by which melanin is formed are not completely understood (97, 238). Apparently tyrosine is oxidized to 3,4-dihydroxyphenylalanine as a first step. There is present in most mammals an aerobic oxidase which is concerned with the further oxidation of this compound. In the biological literature 3,4-dihydroxyphenylalanine is often known as dopa and the enzyme catalyzing its oxidation as dopa oxidase. Whatever may be the exact mechanism of melanin formation from 3,4-dihydroxyphenylalanine, some one of its steps is evidently interfered with when the recessive allele of the albino gene replaces its normal allele. It is interesting that albino mutant types, presumably biochemically similar to that in man, are known in many other mammals and in a number of animals of other groups. In fact, elaborate genetic analyses of melanin formation have been made in a number of these. These studies are summarized in the following section of this review.

Since the publication of the second edition of *Inborn Errors of Metabolism*, two additional defects in phenylalanine–tyrosine metabolism have been described in man. One of these, failure to oxidize phenylpyruvic acid—a condition known as phenylketonuria—has already been mentioned. This condition was first discovered by Fölling (98) and has since been studied by Penrose and others (130, 231). Like alcaptonuria and albinism, this condition is inherited as a simple recessive character. As has already been pointed out, a most unfortunate

and apparently invariable symptom of phenylketonuria is idiocy or imbecility. If phenylpyruvic acid is fed to a phenylketonuric under conditions in which it would be oxidized by a normal person, an increased phenylpyruvic acid content of the blood and urine is observed. The same is true if phenylalanine is fed. No difficulty is encountered in the oxidation of tyrosine by persons suffering from this disease. The assumption is that the normal allele of the gene for phenylketonuria is concerned with the reaction by which phenylpyruvic acid is oxidized to its para-hydroxy analogue.

Phenylalanine is usually assumed to be directly oxidized to tyrosine. It is possible that this oxidation is sufficiently similar to that of phenylpyruvic acid to its para-hydroxy analogue to be catalyzed by the same enzyme and therefore to be controlled by the same gene. If these assumptions were correct, one would expect a phenylketonuric to be unable to make tyrosine from phenylalanine and consequently to require tyrosine as an indispensable component of the diet. An alternative mechanism for the conversion of phenylalanine to tyrosine (suggested by Dr. H. K. Mitchell, personal communication) involves conversion of phenylalanine to its keto acid analogue, oxidation of this in the para-position, followed by reamination to tyrosine. In this case, too, phenylketonurics would require dietary tyrosine. Experimental evidence on the dispensability of tyrosine in the diets of phenylketonurics might therefore be most illuminating. These considerations suggest that the reason mammals have not lost the ability to oxidize phenylalanine to tyrosine during the course of their evolutionary specialization may be that the reaction by which this is accomplished plays a second rôle which, if not exactly that indicated, may still confer a strong selective advantage. This type of relation may well be of general importance and may in many instances account for the fact that from "indispensable" dietary compounds, organisms retain the power to make those "dispensable" ones that occur regularly in the diet in amounts sufficient to meet the needs of the organisms.

Medes (205) has reported studies on a man who was apparently unable to carry out the rather curious oxidation of *p*-hydroxyphenylpyruvic acid to its 2,5-dihydroxy analogue. Ingested precursors of *p*-hydroxyphenylpyruvic acid led to increased excretion of this substance in the urine in a manner analogous to their action in alcaptonuria. From a scientific standpoint it is unfortunate that this disease, known as tyrosinosis, has been reported in only a single individual. We therefore have no way of knowing about its inheritance, although by analogy we might expect it to result from homozygosis for a single mutant gene.

A summary of phenylalanine–tyrosine metabolism in man is given in figure 1. The supposed interrelations of the known and suspected naturally occurring relevant compounds are shown here, based on an interpretation by Haldane (130). This is by no means the only possible scheme so far as details are concerned. The facts and interpretations on which this scheme is based illustrate several points of significance to biochemical genetics. Gene action can be interpreted in terms of a one-gene–one-reaction hypothesis. In one case a specific enzyme is known to intervene between gene and character, and in the others a

similar assumption is compatible with the facts. The use of specific genetic blocks in working out the course of metabolism is also well illustrated. Not only can we say that the existence of the four defective types helps us to understand the system of reactions but, if additional types were available, the course of the process could be described in more detail and with more confidence.

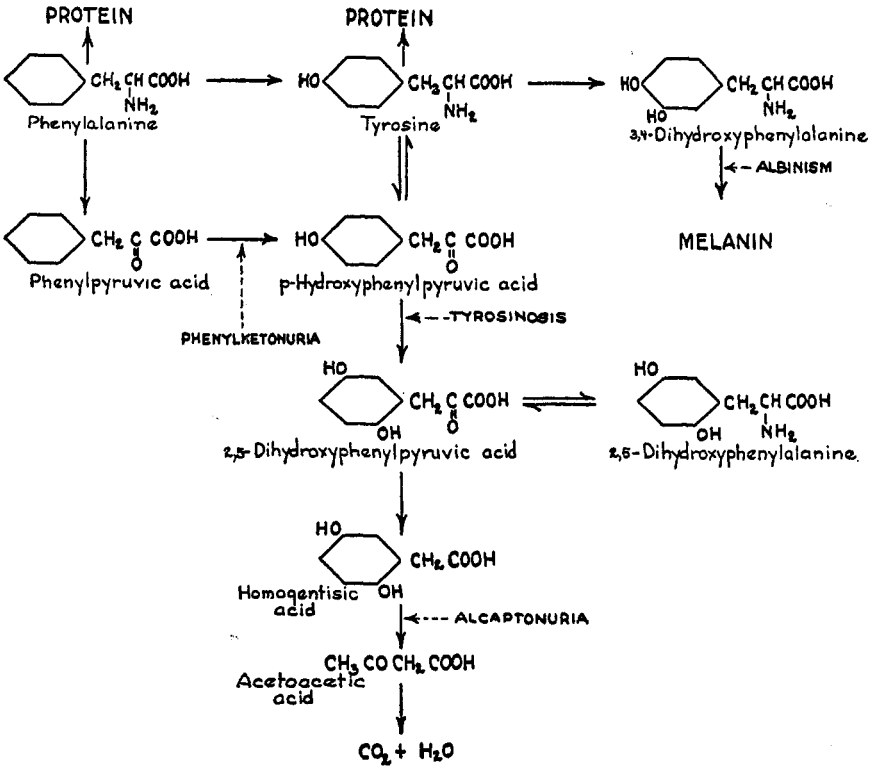


FIG. 1. Scheme of phenylalanine–tyrosine metabolism in man (based on interpretation given by Haldane (130)).

F. The biosynthesis of melanin in mammals

The pigments responsible for the coat colors of mammals are melanins. The familiar variations in such animals as chickens, horses, cattle, and man (skin and hair color) are for the most part genetically conditioned and result from both quantitative and qualitative modifications of the melanin pigments. The actual pigments are largely confined to special ameboid cells known as melanophores. In the birds and amphibia these are known to originate during embryonic stages in the neural crest, a tissue lying just under the ectoderm and over the nerve tube. The evidence for mammals is less conclusive (348), but there appears to be no reason for doubting that here, too, potential pigment cells arise in the neural crest. During development the melanophores migrate from the

neural crest to various parts of the body. Their path of movement is mainly just beneath the ectoderm. In the fishes and amphibia and in general in the case of "skin" pigmentation the melanophores migrate to their definitive positions and there produce pigment in the form of intracellular granules. In hairs and feathers, on the other hand, the melanophores migrate to the hair and feather follicles, where they physically discharge pigment granules into the developing hairs or feathers. In the fowl, where this process has been studied in detail by Dorris, Eastlick, Willier, Rawles, and others (see review of Du Shane (78) for references and additional details), it is known that the life span of pigment cells, the type of pigment they produce, their rhythmic response to local conditions as seen in the barred rock, for example, and other characteristics are subject to their own genetic constitutions.

Essentially similar conditions are found in the amphibia, where Du Shane (77), Twitty (325), and others have shown that the migration of pigment cells, their distribution patterns, and their capacities to produce pigment are subject to genetic control. It is known in these animals that whether or not a melanophore actually produces pigment depends on the constitution of the overlying ectoderm. Thus, the white axolotyl has melanophores which ordinarily produce little or no pigment but which can be induced to do so by experimentally placing them in the proper relation to an ectoderm of the proper genotype (77). In culturing salamander melanophores *in vitro* Twitty (325) has observed a negative correlation between cell motility and pigment formation. This relation may play a significant part in pigment pattern formation in these animals.

Of the mammals the guinea pig is perhaps best known from the standpoint of the genetics of coat color. This is largely due to the efforts of Sewall Wright, who has contributed to our knowledge of the subject over a period of some thirty years. (For details and references beyond those given here the reader is referred to Wright's general reviews (346, 347, 348).)

There appear to be two primary pigments in the guinea pig, both melanins. One of these, the xanthic pigment, is characteristic of red and yellow coat color. The other, known as melanic pigment, is found in black, sepia, and brown animals. Because of general limitations in our knowledge of the chemical constitution and behavior of melanin pigments, it is an open question whether or not these two types of pigment differ in chemical structure. Their absorption spectra appear to differ significantly (7, 109), but this may possibly represent only a difference in colloidal state.

In control of qualitative and quantitative aspects of pigment formation, the distribution of pigmented hairs on the animal, and the distribution of pigments within individual hairs, there are seven known major genes. The albino series of alleles of which five members are known is evidently homologous with the albino genes known in other mammals. This series is concerned with the quantity of both pigments and evidently concerns some process common to the formation of both xanthic and melanic pigments. The normal allele of this gene, *C*, is completely dominant to other known alleles. Intermediate members, on the other hand, show incomplete dominance over less active alleles. The

lowest member of the series, c^a, is essentially an amorph; animals homozygous for it are devoid of pigment under ordinary conditions. Russell's tests for enzymes (254) in frozen skin sections using 3,4-dihydroxyphenylalanine as a chromogen were negative for albino animals regardless of the residual genotype. It is suggested by Wright that the *C* series of alleles is concerned with the quantity or activity of the two enzymes concerned with the two pigment reactions (figure 2), although he recognizes the possibility of alternative interpretations.

The *P* gene appears to be concerned solely with the melanic pigment system. It is completely dominant. In *ff* animals no sepia pigment is formed in the absence of a *P* allele, i.e., in *pp* animals.

While the *Ff* alleles seem to be concerned primarily with the xanthic pigments, *F* can partially replace *P* under certain conditions. In *pp* animals with an active allele at the *C* locus, some sepia pigment is formed in *FF* and *Ff* animals but none is detectable in *ff* individuals. Wright (350) therefore suggests that *P* and *F* act on partially equivalent steps in the formation of melanic enzyme (enzyme IIa or IIb of figure 2). With respect to its relation to xanthic pigment, *F* is complementary to *C*. Both must be present for the full amount of yellow pigment to be formed. If *C* is replaced by a completely inactive allele, no pigment is produced regardless of the condition of *F*. If *F* is replaced by *f* in the presence of *C*, on the other hand, yellow pigment is reduced but not entirely abolished. The *f* allele may be acting as a hypomorph rather than as an amorph.

Genes *B* and *E* are interpreted by Wright (348, 350) as having to do with the differentiation of hair follicles. These are postulated to be of three types, as indicated in figure 2, and they are assumed to determine the course of melanin formation along three corresponding pathways. Gene *E* determines that the follicles will be of either of the two melanic types, while gene *B* determines that a melanic-type hair follicle will be of the sepia rather than the brown subtype characteristic of *E bb* animals.

The gene *A* (for agouti pattern) is concerned with a blocking of melanic pigment differentiation in local regions of individual hairs. Instead of black or sepia there appears red or yellow pigment in a characteristically localized segment of each hair.

The two types of pigment, xanthic and melanic, appear to be competitive in their formation, as though they were derived from a common precursor limited in amount. Thus, black pigment is not simply superimposed on a normal quantity of yellow in a black animal but is laid down at the expense of some of the yellow pigment.

There is an incompletely recessive mutant allele, *s*, which in contrast to its normal allele results in pigment appearing in sharply delimited areas of the body. In the main it has little or nothing to do with the type of pigment formed but somehow has to do with its distribution (351). The mechanism by which one area of skin differs from another is not known, but it is clear that the differential is established during embryonic development of cells. It is suggested (348) that spotting may be concerned with locally arrested pigment cell migration or with a threshold condition in embryonic cells that determines whether pigment cells persist or not.

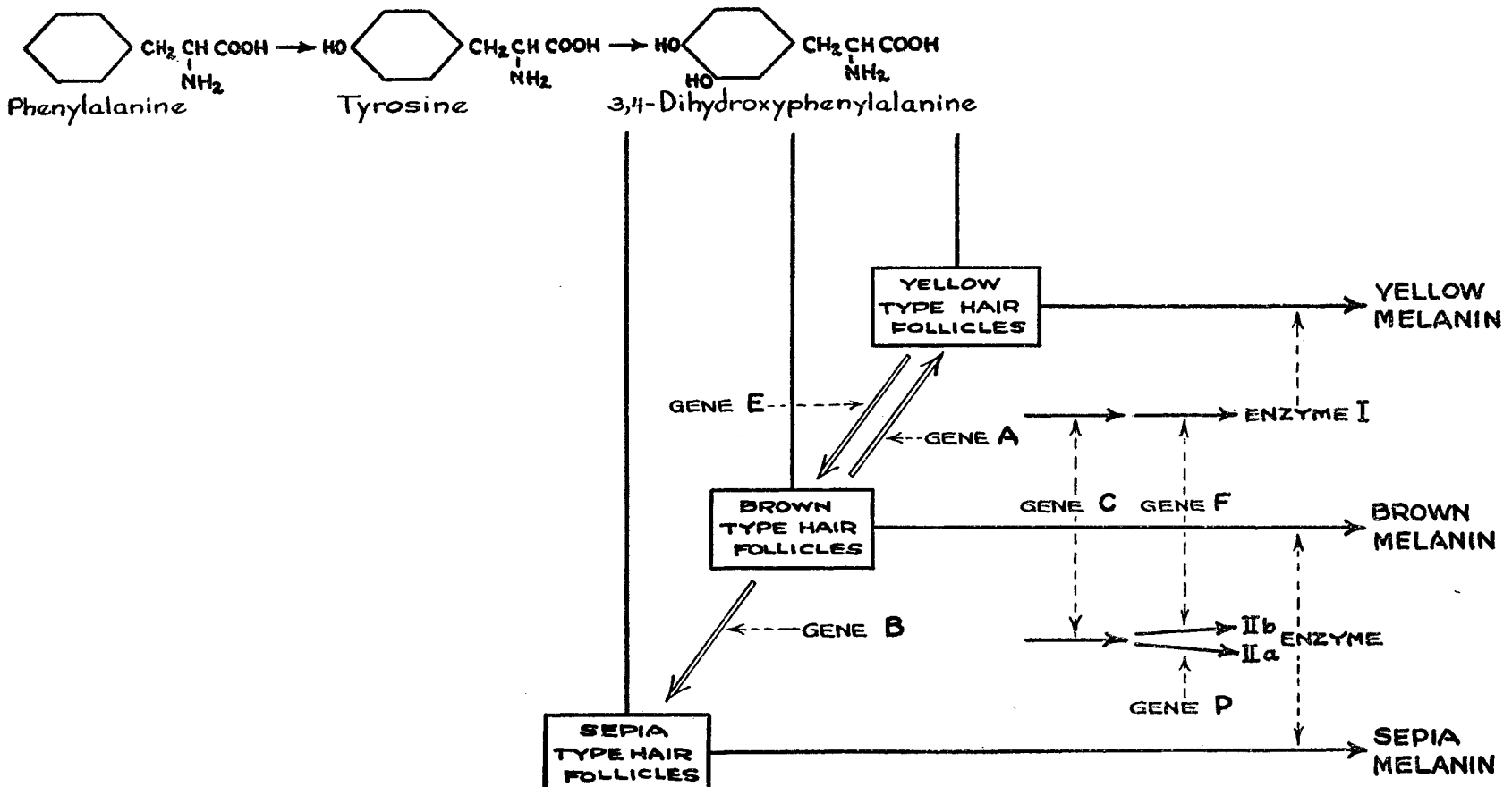


FIG. 2. Scheme of melanin synthesis in the guinea pig (based on work and interpretation of Wright (346, 347, 348, 350)). It is not certain that 3,4-dihydroxyphenylalanine is the common pigment precursor.

Wright (348) has shown that the formation of melanic pigments is subject to temperature modification. If the skin temperature is lowered, albino animals develop some "sootiness," particularly in the extremities, in which the mechanism for control of body temperature is relatively less effective. A similar situation has long been known in the Himalayan rabbit, in which at a low temperature the ears, feet, tail, and snout develop black pigment. Other parts of the body are capable of developing pigment if the skin temperature is kept below 33°C. for a sufficient time. In this and other genetic types of the rabbit Danneel and Schaumann have presented evidence indicating that melanin formation involves a sequence of at least three reactions, the first capable of being suppressed by x-rays and involving the formation of "lipochondria," the second involving aerobic formation of a specific enzyme similar to, if not identical with, dopa oxidase (temperature-sensitive in the Himalayan type and absent in the true albino), and the third, a process of pigment formation requiring oxygen and inhibited by hydrogen cyanide (review, 64).

Siamese and Burmese cats are genetically analogous to the Himalayan rabbit and are physiologically similar in being more heavily pigmented in exposed parts subject to lower temperatures during pigment development (316).

Methods for the quantitative estimation of pigments in the guinea pig have been developed by Russell (252) and Heidenthal (131), following earlier work by Durham, Gortner, Einsele, and others (348). Working with Wright's genetically known material, these investigators have made extensive measurements of the pigments present, particularly in relation to substitution of various alleles at the *C* locus. On the basis of these measurements and his own genetic analysis, Wright has elaborated a formal scheme which, when qualified with suitable rate constants and in other ways, gives remarkable quantitative agreement with observed pigment levels. A much reduced version of this scheme is given in figure 2. In connection with the development of this interpretive scheme Wright has done much pioneer work in the development of theories of gene action. For anyone interested in the details of this development it is necessary to consult the original papers (344, 346, 347, 348).

The interpretation on which figure 2 is based assumes that a single gene may be concerned with the production of two different enzymes. This, however, has not been demonstrated beyond doubt. It seems possible that enzymes I and II may be essentially similar and that the difference in the end products may depend on the conditions under which they act, e.g., type of hair follicle in which pigment is produced. More data are needed before the scheme depicted can be interpreted in detail in terms of enzymes and specific chemical reactions.

While the difficulty of working with melanin-forming reactions *in vitro* has been a serious drawback in the analysis of the guinea pig coat colors, it is nevertheless remarkable how far biochemical genetics has been able to go in this case. It is perhaps worth indicating that it is easy to underestimate the general significance of studies of this kind. Embryologists have often said, "We are not interested in the color of the hair of a guinea pig—this is trivial; what we

want to know is how and why does the eye differentiate?" It is true that the pigmentation of the hair is not of vital importance to the welfare of the guinea pig—but it is not trivial. It is precisely because its presence is not of vital importance to the animal that we can learn so much about its formation and differentiation. Gene substitutions which alter the system are not lethal but leave the organism intact for study. Furthermore, there is no reason whatever to suppose that genes that concern non-vital phenomena differ in any significant way in their manner of action from those related to processes with which the organism cannot dispense. In fact, as we shall see, there is positive evidence that gene action is fundamentally the same in both types of processes.

G. Eye pigments in insects

Without doubt the most complete analysis of any pigment system from a genetic standpoint is that of eye colors in the vinegar fly *Drosophila melanogaster* (32, 216). Mutations of some twenty-five different genes are known to modify the final eye color from the deep red of the wild-type fly. It has been deduced from observations on interactions of different mutant types that there are two independent pigments (198, 343). One of these, a brown pigment, can be removed by gene substitution at any one of four gene loci, leaving only the red component. The resulting eye color is bright red. The red pigment can be removed by replacing the normal allele of the gene *bw* by an inactive allele. This leaves only the brown component. Both pigments can be blocked by a single gene change at the white locus. The physiological interpretation of these facts is that there are two reaction chains leading to brown and red pigment components, but having at some stage some step in common (198, 343). It is this common step that is dependent on the normal allele of the white gene.

The chemical nature of the two pigment components has been studied by Becker (23), by Clancy (49), and by Ephrussi and Herold (90). The brown pigment, known under Becker's name "ommatin," is widely distributed in insects and appears to be a compound of low molecular weight. As will be indicated below, it seems always to be dependent for its formation on the intervention of tryptophan derivatives. It is not soluble in water or in the usual organic solvents, but is soluble in acidified alcohols. It is a redox and pH indicator, is probably bound to a protein *in vivo*, can be benzoylated, and shows a color change in the presence of mineral acids. The red pigment is similar in being of low molecular weight, in probably forming a protein complex, and in showing pH and oxidation–reduction color changes. It differs in solubility, in being independent of the tryptophan reactions, and in not being benzoylated. By the use of absolute methyl alcohol acidified with 1 per cent of dry hydrogen chloride as a solvent for brown pigment and 30 per cent ethyl alcohol taken to pH 2.0 with hydrogen chloride for the red pigment, accurate methods for the quantitative measurement of eye pigments have been developed (49, 90).

The development of the brown pigment component has been shown by Sturtevant and by Ephrussi and Beadle to be dependent on substances capable of diffusing from one part of the body to another (89). These hormone-like sub-

stances have been identified through the efforts of several workers as tryptophan derivatives. Their postulated relations to the brown pigment and to the gene-controlled reactions leading to its formation are indicated schematically in figure 3. Dietary tryptophan is the fly's initial precursor of the two postulated hormones (315). This is converted to alpha-oxytryptophan through a reaction controlled by the vermilion gene (37). A further oxidation to kynurenine occurs. (The Kotake formula of kynurenine has recently been shown to be

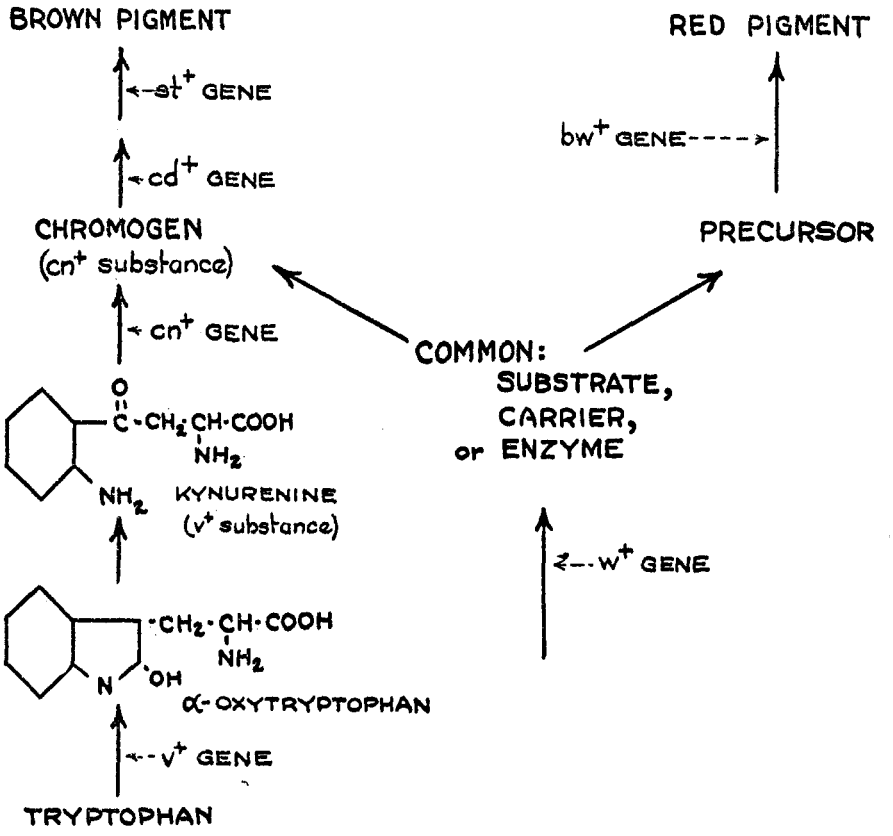


FIG. 3. Representation of insect eye-pigment development

incorrect by Butenandt *et al.* (38).) This is the so-called v^+ substance of Ephrussi and Beadle. This is still further oxidized to the cn^+ substance, which Kikkawa (149) believes to be the chromogen of the brown pigment. The transformation of kynurenine to cn^+ substance is subject to the action of the normal allele of the cinnabar gene. Its recessive allele, when homozygous, results in failure of this action.

Other gene-controlled reactions must contribute to the sequence leading to brown pigment, for it is known that this pigment fails if either the scarlet or the cardinal gene mutates to an amorph. These genes must intervene after the

function of cn^+ substance or in a parallel reaction chain, because flies of both mutant types produce both kynurenine and cn^+ hormone.

Little is known about the reactions by which the red pigment is produced, except that it is dependent on the activity of the brown gene. Since the synthesis of both pigments can be blocked by a change at the white locus, it is essential to postulate a common step in the formation of the two pigments. The nature of this common step remains unknown, however.

The manner of action of the eye-color genes not shown in figure 3 is not known. Some of them influence the quantities of one or both pigment components. In the case of sepia there is apparently a qualitative change of the red pigment (198), but the nature of the modification is not clear. The suppressor-of-vermilion mutant type involves a change in a sex-linked gene. In the homozygous condition this gene somehow operates to restore brown pigment to flies which are homozygous for the vermilion gene and which normally lack brown pigment (18).

Somewhat similar genetic and biochemical antecedants to eye-pigment deposition are known in several other insects. Thus, in the silkworm *Bombyx mori* the white-I mutant type corresponds physiologically to the cinnabar type of *Drosophila* (149). The mutation responsible for blocking the transformation of kynurenine to + chromogen (cn^+ substance) presumably involves a gene homologous to the cinnabar gene of *Drosophila*. The ivory mutant type of the parasitic wasp *Habrobracon* likewise corresponds in both respects to the cinnabar gene of *Drosophila* (16). The meal moth *Ephestia* has black eyes. A red-eyed mutant type found by Kühn and known to be a monogenic recessive was first shown by Caspari (40) to be differentiated by the absence of a diffusible substance, the so-called a^+ hormone. This is now known to be kynurenine (37). The *Ephestia a* allele appears to represent a mutation parallel to that giving rise to the vermilion allele in *Drosophila*. Historically, work on the a^+ hormone of *Ephestia* antedated that on the hormone of the corresponding eye color of *Drosophila*.

It is interesting to observe that as our knowledge of eye pigments of insects and their genetic control has increased, hypotheses concerned with the manner of gene action have become increasingly specific and in certain respects simpler than their forerunners. The facts are certainly not incompatible with the thesis that to every gene it is possible to assign one primary action and that, conversely, every enzymatically controlled chemical transformation is under the immediate supervision of one gene, and in general only one.

H. Anthocyanins and related plant pigments

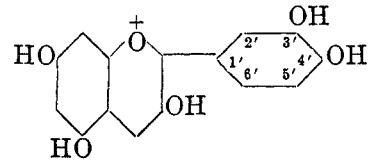
Most of the water-soluble red, blue, and yellow pigments found in flowers and other parts of plants are anthocyanins or related compounds. More than thirty years ago Wheldale (who later wrote under the name Onslow) appreciated that the intraspecific variation with regard to these pigments offered an unusual opportunity to relate genes to the specific structure of the pigments dependent on their activity (332). With subsequent advances in our knowledge

of the chemistry of these pigments due to the activities of Willstätter, Karrer, the Robinsons, and others, it has become possible for biochemical geneticists to make really substantial progress along the lines established by Wheldale.

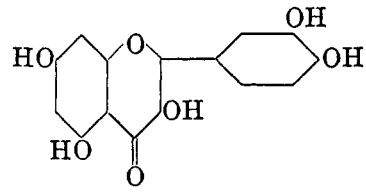
Flowers owe their colors to five types of pigments:

(*a*) *The carotenoids*, not sap soluble, and usually confined to plastids.

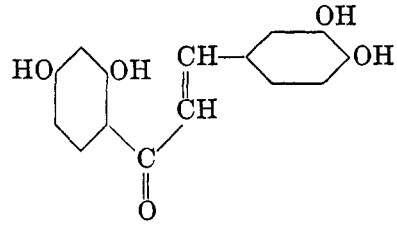
(*b*) *Anthocyanins* (anthocyanidin glycosides): Cyanidin is a common anthocyanidin type and has the structure:



(*c*) *Anthoxanthins and derivatives:* Quercetin is a commonly occurring anthoxanthin. It has the structure:



(*d*) *Chalcones*, probably usually as glycosides: Butein belongs to this group of pigments. Its structure is



(*e*) *Flavocyanins:* These are water-soluble, anthocyanin-like, yellow pigments, the structures of which have not been completely established. Nudicaulin, found in species of the poppy (*Papaver*), is an example (93, 237).

Although several of these pigments have been synthesized *in vitro*, the mechanism by which plants make them is not definitely known. Perhaps the most widely accepted theory is that of Robinson (170, 248), which postulates that both anthocyanins and anthoxanthins have a common origin in the union through aldol condensations and dehydrations of two hexose units and one triose unit. The hypothetical intermediate shown in figure 4, and here designated as the Robinson precursor, is believed to result. From this, cyanidin can be derived through oxidation at carbon atom 1, dehydration between carbon atoms 2 and 3, and ring closure. On the other hand, oxidation at 1 and 3 or at 2 and 3, followed by ring closure, would give quercitin. It is evident that the

Robinson theory can readily be extended to include the formation of chalcones. Through reduction at position 3′, cyanidin gives rise to pelargonidin, while oxidation at 5′ gives delphinidin (figure 4). Analogous modifications of quercitin are known.

So-called leucoanthocyanins occur naturally and have been postulated by Robinson (171, 248) to act in special cases as anthocyanin precursors, for example, in autumn coloration of leaves. A commonly occurring leucoanthocyanin, the structure of which is not known, gives rise to cyanidin on treatment with hydrochloric or sulfuric acid. Bancroft and Rutzler (8) ascribe a more general rôle to leucoanthocyanins in anthocyanin biosynthesis, but their evidence is not wholly convincing. It seems clear that further investigation is

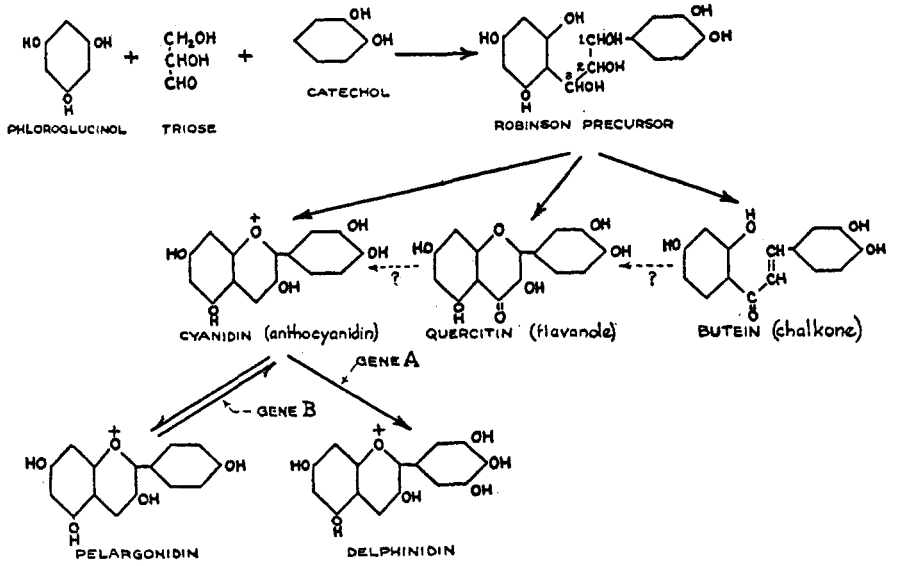


FIG. 4. Interpretation of development of anthocyanin and related pigments (based on Robinson (248) and Lawrence and Price (170)).

necessary before the part played by the leucoanthocyanins can be precisely defined.

Flower pigments are modified genetically in several important ways. In the first place, their presence is determined by the genotype. Thus in the Chinese aster *Cheiranthus cheiri* the dominant gene *Y* is necessary for the appearance of a carotenoid pigment in the petal plastids (265). The water-soluble pigments may or may not be superimposed on this. In a similar way a dominant gene is known to be necessary for the presence of an anthoxanthin in the primrose *Primula acaulis* (170). In the sweet pea *Lathyrus odorotus* complementary dominant genes are necessary for anthoxanthins, i.e., if either of these is present in homozygous recessive form, no anthoxanthin is formed. The presence of anthoxanthin is not dependent on that of anthocyanin. On the assumption that these two genes sponsor different reactions, this would imply that at least

two reactions intervene between the common precursor and anthoxanthin, a conclusion that does not seem unreasonable from a chemical point of view.

The chalcone butein has been isolated from the petals of *Dahlia variabilis*, where its presence is dependent on the dominant allele of a specific gene (236). It has likewise been isolated from *Coreopsis species* and from *Cosmos sulphureus*, where it occurs as the glycoside coreopsin (103, 104, 105, 106). The chalcones as flower pigments have only recently been investigated. Whether they show genetically controlled chemical variations analogous to those of the anthocyanins and anthoxanthins remains to be determined.

In several species the presence or absence of anthocyanins is genetically determined and seems to be qualitatively independent of the presence or absence of anthoxanthins and chalcones. In *Cheiranthus*, *Lathyrus*, and stocks (*Matthiola incana*) the presence of anthocyanins depends on two complementary factors. In flax (*Linum usititissimum*) three complementary factors are known to be necessary for anthocyanin formation. If in these cases the genes concerned affect only anthocyanin synthesis, they must concern reactions by which the common precursor (figure 4) is transformed into the final pigments. This appears to be the situation in *Lathyrus* at least (21). In both the Japanese morning glory (*Pharbitus nil*) and the snapdragon (*Antirrhinum*), on the other hand, genes are known which simultaneously control both anthocyanin and anthoxanthin pigments (21). If in either case the recessive allele is substituted, neither type of pigment is formed. These genes may control some reaction by which a common precursor is synthesized or control the specificity of an enzyme common to the synthesis of both anthocyanin and anthoxanthin.

In the above-mentioned instances, presence of pigment is genetically dominant to its absence. Presumably the active forms of the genes are necessary. There are so-called dominant whites known in a number of forms, for example, in *Pharbitus nil* (170). The manner of action of such dominant "inhibitors" of specific chemical reactions is not known, but it is possible to devise plausible hypotheses to account for it.

If anthocyanin pigments are produced, they may be genetically modified in various ways. One of these ways is in the degree of oxidation of the prime ring. Types in which hydroxyl groups occur at positions 3′ and 5′ in addition to those always present at 4′ (delphinidin types) are usually dominant to those oxidized at positions 3′ and 4′ (cyanidin types) and to those oxidized only at 4′ (pelargonidin types). Cyanidin types are usually dominant to pelargonidin types. Beale (20) has tabulated the predominant direction of mutation for the wild type in a number of species where this is known. This turns out to be from dominant to recessive, i.e., from more oxidized wild-type pigments to less oxidized mutant-type pigments. In two genera, *Lathyrus* and *Streptocarpus*, the interaction of two pairs of alleles concerned with these oxidative differences is known. It is of the following type:

Genes present	*Pigment type*
A B	Delphinidin
A b	Delphinidin
a B	Cyanidin
a b	Pelargonidin

This type of interaction is not consistent with any simple scheme in which the pigments are synthesized in series from less oxidized to more oxidized, or the reverse. It does, however, agree with the scheme of figure 4 on the assumption that gene *A* conditions the presence of an oxidase specific for the cyanidin configuration, while gene *B* determines the presence of an oxidase specific for that of pelargonidin. *A* leads to oxidation at the 5′-position, while *B* does so at the 3′-position. If the cyanidin type is the first formed, as the evidence seems to indicate, it is necessary to suppose that the oxidase corresponding to gene *B* acts to reverse reduction at position 3′, otherwise postulated to occur spontaneously.

Glycoside formation is known to be genetically controlled in two instances, *Verbena* and *Streptocarpus* (170). The situation in *Verbena* is simpler; the 3,5-dimonoside type differs genetically from the 3-monoside type of anthocyanin.

Methylation of hydroxyl groups 3′ or 3′ and 5′ is possible. This is dependent on previous oxidation at these positions and is related to glycosidal type as well. The genetic basis of differences in methylation appears not to be simple in *Streptocarpus*, where it has been studied (170, 172).

Another source of variability of flower color lies in the phenomenon of "copigmentation." Anthoxanthins, tannins, and possibly other compounds may form weak addition complexes with anthocyanin pigments. Such complexes have their color deepened more than would be expected on a simple additive basis. Insofar as presence as well as types of anthoxanthins are dependent on the genotype, copigmented types may be differentiated from those not so modified by a single gene change (170).

Since anthocyanins are pH indicators, variations in the hydrogen ion of the cell sap in which they are found would be expected to modify their color spectra. Such variation is known in at least half a dozen species (170), and is further known to be dependent on the genotype. In all cases more acid petal-cell sap is dominant to the less acid type. The difference is of the order of one-half to one pH unit, and it is interesting that the difference is strictly localized in the petals.

While the data are not yet sufficient to enable one to formulate an interpretation that it is beyond question, they do indicate that relations somewhat similar to those portrayed in figure 4 must exist. The scheme may have to be modified in many details. Furthermore, it is not certain that one simple scheme will apply to all plants. For example, in maize the gene pair *Aa* (see page 22), in the presence of suitable alleles of other genes, differentiates between anthocyanidin (cyanidin-3-glucoside) and corresponding anthoxanthin (quercitin-3-glucoside). Genotypes carrying *A* (*AA* and *Aa*) have the cyanidin derivative in the stems, leaves, husks, and other parts of the plant, while the homozygous recessive form (*aa*) contains the corresponding quercetin derivative (257). This situation has been used as a basis for the argument that the anthocyanin nucleus is derived from that of anthoxanthin through reduction (8, 257). Lawrence and Price (170) and the Robinsons (249), however, are not convinced by this argument.

The hypothesis of a common precursor for the three related water-soluble pigments derives its main support from the observed fact that in *Dahlia*, *Lathy-*

rus, and other forms there is an inverse quantitative relation between anthoxanthin and anthocyanin pigment types. If one is increased through gene substitution, the other is observed to decrease. Apparently a similar competitive relation holds for pigments of the anthocyanin and chalcone types (170). These relations seem most simply explained on the assumption that a common precursor, limited in amount, serves in the formation of all three types of pigment (172, 248). It will be recalled that analogous relations are found in the coat colors of the guinea pig and in eye-color pigments in *Drosophila*.

While there remains much to be learned about flower pigment biosynthesis and its relation to gene systems, it is nevertheless clear from the gratifying progress that has already been made that in many instances genes act in very specific ways. The assumption that each gene functions in a primary way in the control of one specific chemical reaction is certainly strongly supported by work in this field.

I. Disease resistance

Many non-infectious diseases are known to result from deviations in genetic constitution. In a number of these something can be said as to the nature of the correlated metabolic disorders. Several of these concerned with tyrosine metabolism in man have already been mentioned, as has a specific endocrine deficiency responsible for dwarfness in the mouse.

Amaurotic idiocy (Tay-Sach's disease), in some cases at least, appears to be differentiated from normal by a single recessive gene confined largely to members of the Jewish race (326). Clinically it is recognized by gradual development of blindness, idiocy, and paralysis. Metabolically it is characterized by excessive deposition of lipids in the ganglia and glia cells of the brain, spinal cord, and retina (27). Evidently some specific defect in lipid metabolism is responsible.

Defects in porphyrin metabolism are known in man. In phorphorinuria, a rare recessive trait, the urine is characteristically red due to porphorin pigments. Porphyrins are present in exposed tissues where they result in photosensitization. Exposure to light results in bulbous eruptions which usually lead to severe scarring and disfigurement (55, 101). A more serious difficulty of the same general nature is found in xeroderma pigmentosum, although the nature of the sensitizing substance is not definitely known. In this hereditary error of metabolism, epitheliomata or, less often, round or spindle-celled sarcomata develop at the site of skin lesions. The disease is semilethal; some two-thirds of those affected die before the age of fifteen years, while only occasionally a sufferer lives to reproduce (55). Xeroderma pigmentosum is incompletely recessive, as shown by the fact that heterozygotes usually exhibit a type of heavy freckling which, unlike certain other types, is not associated with red hair.

Cystinuria, the excretion of 0.4 to 1.0 g. of urinary cystine per day, pentosuria, the excretion of abnormal quantities of the pentose sugar *l*-xyloketose in the urine, and steatorrhea, a defect in fat metabolism leading to the production of "butter stools" and ascribable to a defective functioning of the pancreas, are

all examples of diseases in man in which the difficulty can be ascribed to a particular phase of metabolism. In some cases, each appears to be inherited as a simple recessive trait (27, 101). Although the evidence cannot be said to be unambiguous, diabetes mellitus appears to be markedly influenced by genetic constitution. One of the factors that make this disease difficult to study from the standpoint of genetics is the fact that it often appears relatively late in life, and the classification of individuals with certainty is therefore difficult. The relation of diabetes mellitus to sugar metabolism and insulin is so well known as to require no comment here. Glycogen storage disease, characterized by excessive storage of glycogen in enlarged liver and kidneys, is probably a recessive trait (27).

Even a casual perusal of Cockayne's *Inherited Abnormalities of the Skin and its Appendages* (55), in which over one hundred inherited diseases are listed, Waardenburg's monograph on inherited eye abnormalities (326), Bodansky and Bodansky's *Biochemistry of Disease* (27), and other such works cannot but impress one with the multitudinous ills which defective genes can inspire. The opportunities for advances in our knowledge of human metabolism through studies of these from a biochemical standpoint are almost unlimited. The concept of the genic control of specific biochemical reactions should do much to bolster confidence that such studies can contribute significantly to our understanding of these various errors of function.

Diseases due to nutritional deficiencies at first sight would seem to have little to do with genes. From the standpoint of immediate relation this is often so, but when it is appreciated that nutritional needs are based ultimately on genetic constitution, a more significant relation becomes evident. We are subject to scurvy while the rat is not, because we differ in genetic constitution. The genes in us in immediate control of ascorbic acid synthesis are inactive, while those of the rat function properly. This thesis will be developed more fully in another connection (page 58); it is sufficient here to say that from a genetic standpoint it is possible to have two individuals of a species differing in a single gene one of which requires a given compound in the diet while the other is able to synthesize it from simple dietary components.

In infectious diseases both the genetic constitution of the host and that of the pathogen are significant in determining susceptibility or resistance. This is true for diseases caused by viruses, bacteria, fungi, and protozoa. It is probably also true for diseases caused by metazoan parasites.

In the tobacco mosaic virus disease Holmes (133) has transferred the resistance of the species *Nicotinia glutinosa* to its cultivated relative *N. tabacum*. The transferred resistance is clearly hereditary, although in this particular case it may not be differentiated from susceptibility by one gene only. In a similar way the virus is itself subject to genetic change. Ordinary strains of virus readily infect normal strains of tobacco. But a mutant form known as Jensen's No. 14 strain produces only local lesions (162). The fact that it is a mutant strain is most clearly shown by the fact that it occasionally back-mutates to a virulent strain. Whether we call the virus mutation a "gene" mutation or not

depends on how we regard the virus. In any case the change appears to be analogous to a gene mutation (see page 71). In the case of both resistant host and non-virulent virus strain infections occur but are confined to local lesions that do relatively little harm to the host plant.

An example of genetic differences in susceptibility of a host to a pathogenic bacterium is found in bacterial wilt of Indian corn caused by *Phytomonas stewartii* (331). Resistant and susceptible strains of corn exist and in some cases are genetically differentiated, resistance being dominant. The bacterium is likewise subject to variation. It undergoes mutation to non-pathogenic forms (177), although, since bacteria reproduce only asexually, there is no way of determining whether or not genes are concerned in such modifications. Mutational changes in pneumococci are known to occur from "smooth" (capsulated) virulent to "rough" (non-capsulated) avirulent types and the reverse. Under certain conditions the change from rough to smooth can be accompanied by a change in antigenic specificity and this can be experimentally controlled (6; see page 75). Other bacteria are subject to similar changes in cultural type and virulence (239).

Fungal diseases show similar relations. Rust of maize attacks certain strains but not others. The difference can in certain instances be attributed to a single gene change (197, 244). Analogous differences exist in the susceptibility of onion strains to onion smudge disease (*Collitotrichum circinans*), but here a good deal more can be said about the chemistry of resistance. White strains of onions are subject to the disease but red or yellow ones are not, even when the bulbs are artificially inoculated with the spores of the fungus. The scales of the colored onions contain the polyphenols catechol (1,2-dihydroxybenzene) and protocatechuic acid (3,4-dihydroxybenzoic acid), while those of the white bulbs do not (180, 329). Both protocatechuic acid and catechol are capable of inhibiting the germination of spores of the fungus in solutions at a dilution of about 1:1000, and they are therefore believed to be responsible for the resistance of the colored onions. It is of interest that both of these compounds are related to quecetin, the main pigment of the yellow onion. Red onions contain related pigments not yet completely identified (180). Whether the polyphenols mentioned are precursors of the pigments or breakdown products is not known.

Genetically it is known that pigmented onions may be differentiated from white ones by a single gene pair, pigmented forms being dominant. Yellow differs from red in a separate gene and red is dominant. An additional partially dominant pigment inhibitor gene is known. In the heterozygous form it results in bulbs with pigmented tops (51, 245). It is clear that these genes having to do with pigment synthesis are also concerned with the presence of catechol and protocatechuic acid. If these are precursors of the scale pigments, both the recessive and the dominant genes for white must block the synthesis prior to their formation.

The apple scab fungus *Ventura inaequalis* shows a variation in its pathogenicity toward specific apple varieties that is clearly genetic in nature (146). This is strikingly demonstrated in this sac fungus by the fact that the eight sexual

spores of a spore sac from certain crosses of virulent by non-virulent invariably give rise to four spores like each parent.

Rust and smut fungi are responsible for many diseases of economically important crop plants. They have been shown to exist in many biotypes (genetically homogeneous strains) with characteristic differences in pathogenicity when tested on specific host strains (48). Thus stem rust of wheat has many such biotypes. These undergo hybridization, both in nature and in the laboratory, and as a result gene recombinations giving new biotypes are formed (59, 250). Furthermore, mutation is known to give rise to new strains in this and other rust and smut fungi (47). The plant breeder is constantly attempting, through hybridization and selection, to obtain resistant host plants with otherwise desirable features. We have here an interesting conflict of interest—the wheat breeder endeavors to develop wheat varieties resistant to the existing biotypes of wheat rust, but as soon as this is done, survival of the parasite demands that nature produce through hybridization or mutation new strains of rust capable of surviving on the newly introduced varieties.

A knowledge of the chemical difference between virulence and avirulence as well as that between host susceptibility and resistance would help us to determine what parts are being played by the genes concerned, even though it might not immediately tell us how to establish permanently immune varieties of wheat.

J. Sex phenomena in unicellular organisms

Without doubt the most remarkable series of studies in biochemical genetics is that of the German investigators Moewus, Kuhn, and coworkers on the flagellate *Chlamydamonas*. This work has been reviewed relatively recently by Sonneborn (277) and by Moewus (214). Because of the war, reports are not available for the three years just past.

Chlamydamonas is a unicellular, uninucleate, green, ellipsoidal, biflagellated organism from 5 to 20 microns long. In the free swimming stage the cells are haploid, i.e., the nuclei contain one set of chromosomes. Vegetative reproduction occurs freely under suitable conditions. Some forms are dioecious with gametes of two sex types. These may be morphologically alike or different. Other forms are monoecious with gametes of only one kind. Gametes of different sexes conjugate, if environmental conditions are favorable, giving rise to a diploid zygote. This forms a heavy wall and becomes a zygote cyst. On germination of this, the nucleus undergoes two typical meiotic divisions and four flagellated haploid daughter cells are formed. If the strain is dioecious, two of these are of one sex and two of the other. In monoecious strains any two gamete cells can conjugate; otherwise the life cycle is similar to that in dioecious races.

Chlamydamonas can be cultured in liquid media, either with or without bacteria present, or on agar media. On solid media the cells have no flagellae and of course are not motile. If cells are cultured in liquid in the dark without either sugar or oxygen, they are likewise not motile. In liquid, motility is restored either by light or by a supply of both oxygen and sugar.

Among the species and varieties of *C. braunii*, *C. dresdensis*, and *C. eugametos*, Moewus has shown that there are a series of sex types of differing potencies or valencies. One sex, which in some species is larger, is called female, the other male. Among both male and females there occur lines varying in valence from 1 to 5. Following Hartmann, sex is interpreted by Moewus as "relative' in *Chlamydomonas*. A female of valence 1 will conjugate with a male of any valence. But it will also conjugate with another female if the second female is of valence 5, 4, or 3, in these cases showing a *male* reaction. Similarly, a male can conjugate with another male if it is sufficiently different in valence.

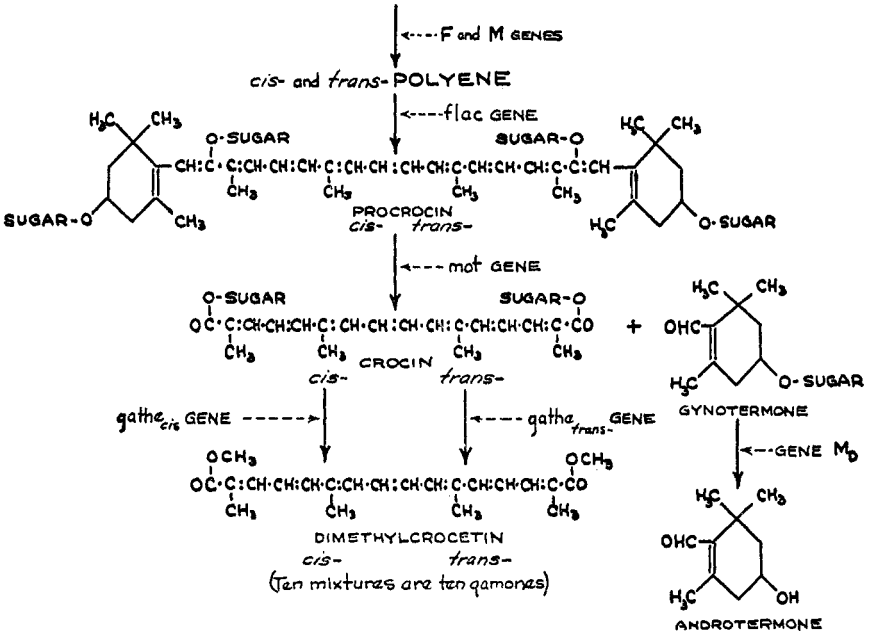


FIG. 5. Scheme of sex-hormone synthesis in *Chlamydamonas* (based on Moewus (214))

Moewus has shown that the cell-free liquid in which active cells have previously been grown contains substances which induce motility of non-motile cells under conditions in which they would otherwise remain non-motile. Such culture filtrates will also determine the sex of non-motile cells of dioecious strains and will induce conjugation in motile cells in which the sex is already determined. Moewus and Kuhn, working with Jerchel, Wendt, and Löw, have established that these filtrate factors are derived from a carotenoid pigment glycoside and have interpreted their formation in a manner summarized in figure 5. It is postulated that from a precursor a polyene is synthesized under the guidance of the genes *F* (for femaleness) and *M* (for maleness). Each of these genes exists in five forms, corresponding to the five degrees of femaleness and five of maleness. The genes *F* and *M* are not alleles of each other but are located some three cross-over units apart. (Crossing over between them can give

types carrying both genes. If the two valencies are equal, these are monoecious lines. Cells with neither F nor M genes are lethal.) Each form of the gene F determines a rato of *cis*- and *trans*-molecules of the polyene as follows:

F allele	*Ratio cis-polyene:trans-polyene*
F^5	98:2
F^4	95:5
F^3	85:15
F^2	75:25
F^1	65:35

In a similar way the five alleles of the M gene determine that the ratio of *cis*- and *trans*-polyene shall be as follows:

M allele	*Ratio cis-polyene:trans-polyene*
M^1	35:65
M^2	25:75
M^3	15:85
M^4	5:95
M^5	2:98

The assumed polyene precursor is converted to protocrocetin as indicated in the scheme. The gentiobioside shown is formed in *C. engametos*, but *C. dresdensis* forms the analogous cellobioside, while in *C. braunii* this is replaced by the cellotrioside. The specific sugar present is determined by which of three alleles of the *flac* gene is present. The ratio of *cis*- to *trans*-isomers determined by the F or M allele present is maintained during protocrocin formation.

Under the control of the gene *mot*, presumably through the mediation of a specific enzyme, protocrocin is cleaved to crocin and picrocrocin or a related compound. Again the ratio of *cis*- to *trans*-forms of crocin is mained. Crocin is the motility hormone. Its activity is astoundingly high, 1.2 molecules per cell being sufficient to cause two flagellae per cell to develop within a period of 20 min. Both isomers are active.

The terminal group glycoside (possibly gentiobioside) formed on the cleavage of crocin functions as the so-called gynotermone. It is capable of determining that the cells of a monoecious culture become females.

Under the influence of two completely linked but not allelic genes, $gathe_{cis}$ and $gathe_{trans}$, two enzymes are produced which oxidize the two isomers of crocin to the corresponding *cis*- and *trans*-isomers of crocetin dimethyl ester. The enzyme which converts *cis*-crocin is active either in light or darkness, but the *trans*-enzyme is active only in the light. In the light, therefore, *cis*- and *trans*-crocetin dimethyl esters are formed in the original ratio determined by the F and M genes. The mixtures of *cis*- and *trans*-crocetin dimethyl esters in the ten ratios indicated above constitute the ten gamones. Each renders cells of corresponding valence capable of conjugation. In the dark only *cis*-crocetin dimethyl ester is formed and this by itself has no gamone activity. It can be converted to the *trans*-isomer through a photochemical reaction, as Kuhn and Winterstein have shown.

In male cells or cells of monoecious strains which carry the gene M_D closely linked to M, an enzyme is formed which is capable of splitting the sugar from gynotermone to give "androtermone" (*l*-4-hydroxy-2,6,6-trimethyl-Δ^1-tetrahydrobenzaldehyde). This substance is capable of determining that cells of monoecious races shall be male in reaction (161). In females the M_D gene is not present, and therefore no androtermone is produced by them.

Aside from the *F* and *M* and *flac* genes, in each of which several alleles occur naturally, the genes indicated in figure 5 were detected by Moewus by inducing mutations in them. Thus, if the *mot* gene is inactivated, both motility hormone and gynotermone fail to appear. That production of both substances is under the immediate control of a single gene is shown by the fact that in all of sixty-four recurrences of the same single-gene mutation, production of *both* hormones was blocked. If the M_D gene is inactivated in cells that would otherwise be males, there result individuals with female termone but male gamone. The $gathe_{cis}$ and $gathe_{trans}$ genes are capable of being independently inactivated through mutation. Strains that cannot convert either *cis*- or *trans*-crocin can be obtained by first inducing a mutation in one *gathe* gene and subsequently causing the remaining one to undergo change.

As regards manner of action, *Chlamydamonas* genes *mot*, M_D, $gathe_{cis}$, and $gathe_{trans}$ appear to control specific enzymes which in turn catalyze specific reactions. The $gathe_{trans}$-controlled enzyme has the unusual property of acting only in light. The *flac* alleles are assumed to work by way of enzymes specific to the type of sugar residue of procrocin. If $flac^{gent}$ is present, the sugar is gentiobiose; if the $flac^{cebi}$ allele is substituted the cellobioside is synthesized; while the $flac^{cetri}$ allele determines that the sugar will be cellotriose. The *F* and *M* series of alleles have still more remarkable properties. It will be recalled that they determine in what proportions the *cis*- and *trans*-isomers of the polyene precursor will be synthesized. How this is accomplished is not known with certainty, but it is proposed by Kuhn and Moewus that the mechanism is possibly similar to the one determining that amino acid residues in proteins will occur in ratios according to the Bergmann–Niemann numbers. While this is by no means a completely satisfying interpretation, it is remarkable that the *cis*–*trans* ratios do fall into a series that can be approximated by Bergmann–Niemann numbers. The agreement is indicated in the following comparison for female sexes (277):

SEX	VALENCE	BERGMANN–NIEMANN NUMBERS	*Cis–trans* RATIO OF DIMETHYL ESTER OF CROCETIN	
			Calculated	Observed to be active
Female	1	2:1	66.7:33.3	67–64:33–36
	2	3:1	75.0:25.0	75–74:25–26
	3	2×3:1	85.7:14.3	86–83:14–17
	4	2×3^2:1	94.7: 5.3	96–94: 4– 6
	5	2×3^3:1	98.2: 1.8	99–97: 1– 3

A similar agreement is found for the five male types with the ratios transposed.

It is most unfortunate that after presenting so beautiful an interpretation

and one which agrees with so many of the reported facts, one must introduce a note of skepticism. The facts reported and the interpretation are almost "too good to be true." As a matter of fact, Philip and Haldane (232) have, on the basis of a statistical analysis of certain of Moewus' genetic results, made just such a criticism. Accepting the interpretation at face value and then examining the distribution of sampling errors, they come to the conclusion that, "... if every member of the human race conducted a set of experiments of this type daily, they might reasonably hope for such a success once in 50,000 million years." Moewus (214) has attempted to reply to this most serious criticism. Pätau (229) has analyzed other data of Moewus with a similar conclusion (see also Ludwig (189)). Sonneborn (277) has subjected the Kuhn–Moewus work to a detailed and searching criticism and concludes that it is most important that the work be repeated by investigators working independently. The writer is in complete agreement with this sentiment.

A second case of sex differentiation in a unicellular organism of particular interest to biochemical geneticists is that found in the ciliate protozoan *Euplotes patella*. Kimball (150, 151) has observed that in this organism, in which the individuals are normally diploid, there are six mating types. Genetic studies show that these types are determined by three allelic forms of one gene. The three homozygotes mt^1mt^1, mt^2mt^2, and mt^3mt^3 represent three types, while the three heterozygotes, mt^1mt^2, mt^1mt^3, and mt^2mt^3, give rise to the remaining three types of mating behavior. Biochemically it is found that culture filtrates contain specific substances which are responsible for conjugation reactions. There are three such substances, each controlled by one of the mating type alleles. The three homozygous types therefore each produce one of these sex hormones, while each of the three heterozygotes produces one of the three possible combinations of two substances. An animal may be activated by any substance other than the one or two specific to itself. Unfortunately, the chemical nature of these substances has not yet been determined. The gene–reaction correspondence is here very striking, and in the apparent independent action of alleles in controlling the production of different specific substances we have a resemblance to the gene–antigen relation to be discussed below.

The ciliate genus *Paramecium* is similar to *Euplotes* in that several interfertile mating types may be present within one species, but it apparently differs in not releasing mating-type substances into the culture medium. There is, however, evidence that specific substances which may be transferred from animal to animal by contact are concerned (278). For further details regarding mating types in species of *Paramecium* the reader is referred to recent papers by Sonneborn (278) and by Jennings and Opitz (142).

K. Genes and immunological specificity

Antigens are known to be substances of high molecular weight (10,000+). They are often proteins, though apparently polysaccharides may exhibit antigenic properties (30, 165). Antigenic specificity may be determined by relatively simple compounds, the so-called haptens of Landsteiner, combined with proteins. An antigen of one species injected into the blood of another under

the proper conditions may induce the formation of an antibody specific to the inducing antigen. Antibodies are often, if not always, serum globulins. The antigen–antibody reaction is the result of an intimate union between the two, probably involving hydrogen bonds and at least under certain circumstances reversible. This union may result in any one of several reactions, such as agglutination, precipitation, lysis, or toxin neutralization. The antigen–antibody reaction may also be detected by complement fixation (reviews: 30, 165).

The antigens characteristic of a species are intimately related to its genetic makeup. This is well illustrated in the blood groups of man. The first of these, the A–B blood groups, were first discovered in 1900 by Landsteiner (164). It has subsequently been determined that the four main groups, 0, A, B, and AB, are genetically determined by the three alleles I^0, I^A, and I^B (reviews: 301, 335). Of these I^0 is inactive, I^A is correlated with antigen A, and I^B conditions the presence of antigen B. Both I^A and I^B are dominant to I^0, but when I^A and I^B are present in the same individual, neither is dominant, but determine that both antigens A and B will be present. The antigens A and B are found in red blood cells and in other tissues; they are known as hemagglutinogens. Antibodies, known as hemagglutinins, are normally present in sera if their corresponding antigens are absent. Summarizing these genetic and immunological relations, we have the following:

BLOOD GROUP	GENETIC CONSTITUTION	ANTIGENS IN CELLS	ANTIBODIES IN SERUM
0	I^0I^0	None	α, β
A	I^AI^A or I^AI^0	A	β
B	I^BI^B or I^BI^0	B	α
AB	I^AI^B	AB	None

The agglutinins α and β correspond to the agglutinogens A and B. If cells and sera with either one or two pairs of corresponding agglutinogens and agglutinins are mixed, agglutination of cells occurs. A subgroup of type A is known, and the antigen characteristic of it is dependent on a fourth allele of the series (335). This, however, does not alter the principles involved in either the inheritance or the serological action.

The M–N blood types, discovered in 1927 by Landsteiner and Levine (166), differ from the A–B groups in that antibodies are not normally present but must be produced through immunization. For this reason the M–N blood types are not clinically important in blood transfusion. The M–N types are determined by the two alleles A^M and A^N, which are independent in inheritance of the A–B alleles. There are three genotypes possible and they correspond to the three blood types as follows:

GENOTYPE	BLOOD TYPE
A^MA^M	M
A^MA^N	MN
A^NA^N	N

Again each allele is related to a specific antigen, and if both are present they act independently, each producing its characteristic antigen. There is no inactive allele of this gene known.

In connection with their early work on the M–N blood types Landsteiner and Levine discovered a hemagglutinogen in man immunologically different from those of the A–B or M–N groups. This has become known as agglutinogen P. Antibodies against it may occasionally occur normally in human sera but usually are not present. The presence of P is inherited as a simple dominant trait. About 24 per cent of the individuals classified proved not to carry the P agglutinogen, i.e., were homozygous recessive for the gene concerned with its production. The available information on the occurrence of the P antigen, its properties, and its inheritance have been summarized by Wiener (335).

A fourth series of blood types in man, first reported by Landsteiner and Wiener in 1940 (168), is designated the Rh series after the Rhesus monkey in which the Rh antigen was first found. According to Wiener (336) there are six alleles of a gene independent of the A–B and M–N genes responsible for the Rh antigenic specificities. One of these, *rh*, is inactive. Three, Rh_0, Rh', and Rh'', directly control the production of three corresponding antigens (see also Murray (220)). The remaining two, $Rh_1(Rh_0')$ and $Rh_2(Rh_0'')$, each produce antigens with the combined specificities $Rh_0 + Rh'$ and $Rh_0 + Rh''$. These various alleles determine antigens detected with special antisera. As tested with standard Rh antiserum, about 15 per cent of the individuals of this country are Rhesus-negative (genetically *rh rh*), while the remaining 85 per cent are Rhesus-positive (*Rh Rh* or *Rh rh*). Again, as in the M–N types, antibodies are not normally present in the serum of Rh-negatives but can be produced through immunization either in man or in other animals. An interesting relation arises when an Rh-negative mother carries an Rh-positive fetus. The Rh antigens of the fetus may, under circumstances not yet clearly understood, leak through the placenta and induce antibody formation in the mother's blood. These antibodies may then pass back through the placenta and lyse the red blood cells of the fetus in the same or a subsequent pregnancy. This situation may lead to prenatal death, to a serious postnatal state of the infant known as erythroblastosis fetalis, or possibly, as recently indicated, to permanent mental impairment (276a). It is only under the specific genetic relations indicated—an Rh-negative mother, an Rh-positive fetus, and of course an Rh-positive father—that erythroblastosis fetalis results. Fortunately, the difficulty arises in only a small fraction of the pregnancies. For further details and references the reader is referred to Wiener's excellent summary of the Rh and other blood antigens of man (335).

A most illuminating study of genetically controlled interspecific variation is that of Irwin, Cole, and Cumley on the pigeons and doves (family *Coumbidea*). Hybrids involving the two species *Columba guinea* (wild pigeon) and *C. livia* (domestic pigeon) and three species of dove, *Streptopelia chinensis* (Pearlneck), *St. risoria* (Ring dove), and *St. senegalensis* (Senegal) have been made in various combinations, and they and their progeny in back-crosses to pure species have been studied with respect to blood-cell agglutinogens and serum antigens

(for reviews and references see 61, 139, 140, 141). In principle these findings can be summarized as follows: For any two species there are both common and species-specific cellular antigens which can be detected with appropriate antisera. Hybrids between the two species have the antigens common to the two plus those specific to both parents. Thus, in the Pearlneck–Ring dove hybrid there may be present cellular antigens A B C D, whereas Pearlneck has only A B C and Ring dove only B C D. A is specific to Pearlneck and D to Ring dove, while B and C are common to both. Back-crosses of the F_1 hybrid to the parental species show that species-specific antigens are inherited as though they were determined by dominant genes. The Pearlneck–Ring dove hybrid back-crossed to Ring dove shows that there are at least ten cellular antigens specific to Pearlneck. Since the genes determining these are heterozygous in the F_1, while the Ring dove parent is homozygous recessive, the back-cross progeny segregate in a one-to-one ratio of presence and absence of each of the ten Pearlneck-specific antigens. The analogous back-cross of the hybrid to Pearlneck is very difficult to make, and accordingly relatively few offspring have been tested. Such tests as have been possible, however, indicate that at least nine cellular antigens are specific to the Ring dove parent (139).

If the cellular antigens of the two species, Pearlneck and Ring dove, are compared with still other species, e.g., *C. livia* and *C. guinea*, it is found that certain of the antigens shared by Pearlneck and Ring dove are specific to *C. guinea* as compared with *C. livia*. Other interspecific comparisons show similar relations.

The study of antigenic differences in *Columbidea* has recently been extended to serum antigens (60, 62). In successive back-crosses of Senegal–Ring dove hybrids to the Ring dove parent, at least three distinct serum antigens have been identified and shown to be inherited in a mendelian fashion. The genes responsible for these appear to assort independently of those determining the several (possibly ten or more) Senegal cellular antigens not present in Ring dove.

Individual chickens are known from the work of Landsteiner and Levine (167), Todd (320, 321), Thomsen (318), and others to vary in their blood-cell antigens. This variation is known to be inherited in a manner essentially similar to that of the blood groups of man. Wiener (335) has pointed out that certain of the data of Todd can be interpreted on the basis of three alleles of one gene, each producing a specific agglutinogen, plus an independent gene pair with a dominant allele controlling the production of a fourth agglutinogen and an inactive recessive allele. Ducks have been investigated by McGibbon (195) and others. The Mallard and Muscovy show relations similar to those found in the pigeon–dove group. Each contains species-specific cellular antigens as well as a group of antigens common to both. Since the hybrid between these species is sterile, direct tests of the hereditary basis of species-specific antigens is not possible. However, genes determining them must be dominant, since the antigens are present in the F_1 generation. Intraspecific antigenic differences are known to be inherited in these species, the presence of an antigen being dominant to its absence.

Wiener (335) has summarized available data on individual variation in cellular antigens in various mammals. Apes and monkeys show blood group variation much like that of man; in fact, homologous agglutinogens can be identified in many instances. There are few or no inheritance data available in these animals. Heritable cellular antigens are known in cattle, sheep, rabbits, mice, rats, and dogs. In each case the presence of a specific antigen parallels the dominant allele of a specific gene. An interesting expression of immunological differences is found in animal tissue transplants. It has long been known that tissue—for example, skin—can be successfully transplanted from one location to another in a single individual, but not usually from one individual to another. Loeb and Wright (186) found this to be so in guinea pigs. But if lines of guinea pigs, inbred by brother–sister matings for a sufficient number of generations to insure that substantially all genes become homozygous, are used, transplants between individuals are successful. If two inbred lines, each of which fails to accept grafts from the other, are crossed, the F_1 hybrid will accept grafts from either parent. The parent lines, on the other hand, will not accept grafts from the F_1 hybrid. These relations are interpreted genetically on the assumption that certain genes are concerned with the success of the transplant and that they act in such a way that an animal will not accept a graft containing dominant alleles which it does not itself have. On the other hand, such dominant alleles in the host are without effect regardless of whether the graft has them or not. Immunologically it has been supposed (127, 307, 349) that these dominant genes are concerned with antigen formation. If a graft contains antigens not shared by the host, antibodies against the transplanted tissue are induced and they interact with the graft antigens in such a way as to cause the graft to be cast off or resorbed. Schematically this situation can be represented as follows:

	GENETIC CONSTITUTION	ANTIGENS IN TISSUES
Line 1	*AA bb*	A
Line 2	*aa BB*	B
Hybrid	*Aa Bb*	A, B

It is readily seen that of the six possible host–donor combinations, in only two (Line 1 on hybrid and Line 2 on hybrid) will the host have all the antigens of the graft. The results in the F_2 generation and in back-crosses will depend on how many antigenic differences exist between the two lines and how they are distributed. By the methods of genetics these relations can be determined. Similar relations appear to hold for tissue transplants in mice (182). In rats the situation appears to be somewhat more complex and has not yet been analyzed on a basis as simple as that just given (185). As will be pointed out later (page 53), essentially similar relations often hold when tumor tissues are transplanted from one individual to another in experimental animals.

In all instances so far presented it appears that a one-gene–one-antigen relation obtains. Actually, while this is the relation in the great majority of cases that have been studied, there are several notable exceptions. In the dove

hybrids—for example, Pearlneck by Ring dove—there appear so-called "hybrid substances," i.e., cellular antigens not present in either parental species (140). Analogous hybrid antigens are found in the Mallard–Muscovy duck hybrid referred to above. Occasionally antigens of an apparently similar nature appear in chicken crosses (318). In the dove and duck hybrids these appear to be the result of two or more complementary dominant genes, one or more contributed by each parent and possibly having to do with species-specific antigens. Thomsen suggests that in the chicken they may represent recessive antigenic determiners that were masked in the parents, but the complementary dominant gene theory would appear to fit the facts equally well. The usual one-to-one gene–antigen relation suggests, as has often been pointed out (129, 130, 140, 347), that antigens may be direct gene products. This, of course, is in agreement with the general working hypothesis of gene action presented earlier in this paper. The antigen, or at least the part of it responsible for its specificity, may well be a direct copy of a gene. The antigens dependent for their production on two or more complementary genes would, on this hypothesis, have their specificities determined by two components, each copied from a different gene.

An interesting biological application of immunological specificities is found in "serological systematics." It is found that the degree of antigen difference between any two species is correlated with their systematic divergence as determined on morphological grounds. On the assumption that antigens in general are gene determined, measurements of serological relationships measure degrees of relationship in terms of a particular category of genes. By and large, these would be expected to give a fair picture of relationships in more general terms. Extensive studies in both plant and animal kingdoms have been made on this basis (reviews: 31, 45) and have contributed information of substantial value to the systematist.

L. Self-sterility in plants

Evidently in most plants and animals there is a strong selection against close inbreeding, for there exist several well-developed mechanisms for preventing its occurrence. One of the most interesting of these from the viewpoint of gene action is that responsible for self-sterility or self-incompatibility. In the early days of plant genetics it was observed that many species of monoecious plants cannot be successfully self-fertilized but can be crossed freely with other plants even though these be relatively closely related. The phenomenon is widespread in the plant kingdom (81). The most prevalent genetic basis of it is the oppositional allele mechanism first worked out for tobacco by East and Mangelsdorf (82). In this and many other plants there exist many forms or alleles of a specific gene, and these act in such a way that a pollen tube (haploid generation) carrying one of these alleles will not grow sufficiently fast to bring about fertilization in a style (diploid maternal tissue) that carries this same allele. Thus an S_1S_2 plant produces both S_1 and S_2 pollen, but neither type will grow in the S_1S_2 style, although both will function perfectly in an S_3S_4 style. In a mating such as $S_1S_2 \times S_1S_3$, half the pollen (S_3) functions. Some fifteen such alleles were

identified in tobacco. In the evening primrose *Oenothera organensis* Emerson (84) has shown that there are at least forty-five such alleles of a single gene. In the clover, *Trifolium repens*, Atwood (3) has found as many as thirty-nine alleles in a relatively small population. Actually there must be many more than this. With random distribution of such a large number of alleles in a population the chance of any two plants being identical in constitution and hence cross-sterile is of course small. Wright (345) has given a mathematical analysis of the distribution, mutation frequencies, etc. of self-sterility alleles in *Oenothera organensis*.

Although it has often been suggested that the incompatibility reaction between style and stigma is similar to an immunological reaction, only a beginning has been made in interpreting self-sterility in physiological and immunological terms (84, 175a). From the standpoint of structure and function of the gene, the remarkable aspect of the self-sterility alleles is that there are so many forms possible of a single gene, each maintaining its autocatalytic property and at the same time imparting a unique specificity to the pollen tubes and tissues of the style. In clover, judging from Atwood's results, one might guess that there are at least a hundred different alleles possible.

M. Genes and cancer

The problem of the nature and causes of cancer is a tremendous one and one on which a voluminous literature has grown up. There is as yet no general solution, but it can nevertheless be said that genes are of basic importance. In one sense, abnormal cell proliferation may be regarded as a defect in normal differentiation. Instead of acquiring a definitive form and becoming a normally integrated part of a specific tissue, cancerous cells retain a high division rate. In this respect they are like embryonic cells, but in other regards they differ, e.g., in usually having a granular cytoplasm, in the appearance of cytoplasmic constituents, and often in irregularities in chromosome behavior.

For many years it has been known that for many transplantable tumors the ability of the tumor cells to survive in the host is dependent on both the genetic constitution of the tumor cells and that of the host into which they are put. Little and Tyzzer (184), Little and Strong (183), Cloudman (54), Bittner (24), and many others have found that the behavior of a mouse tumor graft is subject to the same principle as is a normal tissue graft (page 51). If a tumor arises in a pure line, it can be transplanted to other individuals of the same line but not usually to strains of other genetic constitutions. If two inbred lines, one susceptible to a tumor transplant and the other resistant, are crossed, the F_1 mice are susceptible. In back-crosses to the resistant line a segregation of resistant and susceptible mice is found, the numerical relations of which indicate the number of genes concerning tumor resistance by which the parent strains differ. As in normal tissue transplants, the requirements for a successful transplant are that the tumor tissue have no dominant alleles of pertinent genes which are not also present in dominant form in the recipient. Sometimes the significant

difference may be in only a single gene in which case the situation will be as follows:

Parents (1)	*aa* (resistant)
(2)	*AA* (susceptible)
Tumor (spontaneous in (2))	*AA*
F_1 hybrid	*Aa* (susceptible)
Back-cross F_1 to (1)	1 *Aa* (susceptible)
	1 *aa* (resistant)
F_2 generation	1 *AA*
	2 *Aa* (susceptible)
	1 *aa* (resistant)

Usually strains differ by a larger number of significant genes, often as many as six or eight. If transplants of tumors arising in the F_1 hybrids between strains are made, the F_1 is susceptible but the two parental strains are resistant. The back-cross and F_2 generation segregations indicate the number of genes concerned and agree with one another (303). An immunological interpretation can be applied to these results just as in the case of normal tissue transplants (127). If the tumor carries one or more dominant genes conditioning the presence of corresponding antigens and the host has inactive or immunologically different alleles of these genes, the tumor graft induces antibody formation against itself and it then retrogresses. Several types of transplantable tumors in mice have been shown to behave in this manner (reviews: 26, 181, 275). MacDowell and associates (246), as well as others, have shown that a similar genetic interpretation can be made of differences in susceptibility to transplantable leukemia (cancerous leucocyte-like cells that circulate in the blood stream).

It has been observed that occasionally transplantable tumors in mice undergo spontaneous changes in growth properties. A number of these have been shown by Strong (302), Cloudman (53), and Bittner (24) and others to involve genetic simplification, in that the modified tumors require that fewer dominant genes be present in the host for successful growth than were required by the original tumor. Mutations of this type, which may of course involve physical loss of chromosome segments containing the relevant genes, may have an important bearing on the origin of transplantable tumors which are not strain-specific. If a tumor, originally containing gene-controlled antigens that render it strain-specific, is put in a host capable of building antibodies against it, then such a host will act as an enrichment culture for any mutant tumor cells which have lost antigen-determining genes either through true gene mutation or through physical loss. Loss of all species-specific antigen-genes would of course result in a tumor without any strain specificity. An alternative hypothesis involving masking of antigens through mutant changes has been proposed by Gorer (114).

That there is an immunological basis of tumor specificity has been experimentally demonstrated by Gorer (113), who showed that a sarcoma originating in one strain of mice actually induced agglutinin formation in another strain in

which tumor transplants showed regression. The two strains differ by two or three significant genes, and one of these was shown to be identical with one shown in independent experiments to control the production of hemagglutinogen II. A similar situation appears to obtain with regard to genes for susceptibility to transplantable "stem-cell" leukemia in mice; one of the genes concerned is reported to be identical with the hemagglutinogen II gene (115). Antibodies specifically directed against the Brown–Pierce tumor have been reported in rabbits (147). Reference is made to Bittner (25, 26), Snell (275), and Little and Gorer (181) for further details and reference to the literature on transplantable tumors.

A number of specific compounds are known to increase the incidence of tumor origin. Notable for effectiveness among these carcinogenic substances are 1,2,5,6-dibenzanthracene, 3,4-benzopyrene, 20-methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene (reviews: 36, 181, 275). So far as genetic aspects of tumor induction go, two main questions arise, *viz.*: (*1*) does genetic constitution influence the response of the organism to carcinogens, and (*2*) are carcinogens effective through inducing mutations? A discussion of the second question is deferred (see page 57). To the first the answer is yes. It is known that strains of mice differ in their responses to carcinogenic agents (reviews: 181, 275), but the detailed genetic basis of this difference is not known.

Tumors arising without treatment are known in many animals, both invertebrate and vertebrate. Because of the obvious relation to cancer in man, mammals such as the mouse have been most studied with regard to these.

In the vinegar fly *Drosophila melanogaster* the development of spontaneous benign larval tumors is known to be genetically conditioned (253). In addition, a recessive sex-linked lethal is known in which all males carrying the mutant allele develop melanotic lesions at the femur–tibia junction (117).

Kosswig and Gordon have found that hybrids of the two genera of viviparous top minnows, *Platypoecilus maculatus* and *Xiphophorus hellerii* (Mexican swordtail), having a specific genetic constitution develop melanotic overgrowths (111, 112, 157, 158). The requirement for the development of such melanomas is that the hybrids carry the sex-linked dominant gene for macromelanophores from the platyfish parent. Since in the platyfish parent such melanophores do not undergo excessive proliferation, it is clear that the contribution of the swordtail to the hybrid is important in such unregulated growth. Breeding studies make it highly probable that this contribution consists of partially dominant genes. We have here a clear case of genetic regulation of the growth of a particular type of cell.

Marked variations in the incidence of various types of tumors are known in different inbred strains of mice (reviews: 26, 181, 275). For example, lines are known in which approximately 90 per cent of the females develop breast cancer, while in others the incidence is below 1 per cent. Since the observed incidence varies with age, the values for particular lines are subject to strong modification by environmental factors, e.g., nutritional variations that influence the length of life of a mouse. The difference between high- and low-tumor lines is

clearly subject to genetic control, although the exact genetic basis cannot be determined. It has recently been shown that, in addition to genetic factors, there is a milk-borne agent concerned. Bittner and others have established that this has many of the properties of a virus (9). For the development of this particular type of tumor it is apparently necessary that the milk-borne factor be present and that the genetic constitution of the host be favorable to its multiplication and expression.

MacDowell and coworkers, as well as others (87, 193, 246), have shown that the occurrence of spontaneous leukemia in mice is strongly influenced by genetic constitution. Again, high-incidence and low-incidence inbred lines are known. They evidently differ by at least several significant genes, judging from the results of crosses between them. In addition, there is evidence of extrachromosomal factors, though the nature of these has not yet been established (181).

Similar relations no doubt hold in the occurrence of tumors in man. The hereditary disease xeroderma pigmentosum has already been mentioned. Here, as Haldane (127) points out, it is clear that while exposure to light is evidently the immediate cause of tumor origin, genetic modification is responsible for the abnormal photosensitivity. This relation illustrates the obvious general principle that both genetic constitution and environment are indispensable components of the living system and that it is senseless to argue in any case that one is more or less important than the other. This does not of course deny that a given property of the system may be more susceptible to modification through defined variations in one component than in the other. In the case under discussion there are obvious ways of modifying the end result by varying either a gene or the light exposure.

Neurofibromatosis (von Recklinghausen's disease) is apparently inherited in man as a simple dominant character (55). It is characterized by local pigmentation of the skin, tumors of the skin, and tumors of the peripheral nerves. Most sufferers do not live to reproduce, and consequently the condition is limited to a few generations in a given pedigree. Obviously, to maintain its frequency in the population, it must repeatedly recur through gene mutation. Still other strong predispositions to specific types of tumors in man are known to be inherited (55, 130), and there can be no doubt but that the occurrence of tumors in general has an important genetic component.

From the viewpoint of genetics two questions as to how tumor cells arise are of basic importance. These are: (*1*) how do such cells differ from normal cells? and (*2*) how does the characteristic difference arise? It is possible that the difference is genetic, in which case it must arise through somatic mutation. This hypothesis, the origin of cells with unregulated growth characteristics as a result of localized gene mutation, has been proposed many times. The difficulty is that no one has yet devised a method of directly subjecting it to experimental test. This of course cannot be done by the classical methods of genetics, because the somatic cells in which tumors arise leave no descendents by sexual reproduction. There are, however, a number of indirect arguments that bear on it. It is, for example, consistent with the observation that tumors frequently

arise in local regions, probably in single cells. A second argument is of a deductive nature. If genes do control specific steps in metabolism, there must be genes which serve to integrate cell division in different parts of the organism. Many genes, if not all, are subject to change, through either mutation or loss, and unless those having to do with keeping cell division in check are immune to such a change, it must be possible for unregulated growth capacities to arise through mutant changes in them. If somatic mutations are a frequent cause of cancerous properties of cells, then one might expect the carcinogenic agents referred to above to cause genes in general to undergo mutation. While x-rays and ultraviolet radiation are known to induce mutations, this has not been found to be so in the tests that have been made with carcinogenic chemicals. While this does not disprove the hypothesis, it certainly does not argue in favor of it. From numerous investigations on the genetic basis of antigenic differences, such differences are usually if not always to be ascribed to specific gene differences. It would appear at first thought, then, that the demonstration of an antigenic difference between a tumor and the tissue of the animal in which it arose—or others of the same genetic constitution—would strongly indicate that the tumor had arisen through somatic mutation. There are, however, at least two serious objections to this argument in its simplest form. The first is the possibility that the immunological specificity of the tumor may be due to an abnormal cell constituent such as a virus. This may be so even though the virus cannot readily be obtained free of cells. Of course, such a virus might conceivably arise from a normal cell constituent (see page 84), in which case the tumor origin might still be regarded as a special type of somatic mutation. A second weakness of the argument is that there is some evidence for the existence of organ-specific antigens (330). In the case of the rabbit lens antibody of Guyer and Smith (125, 126), for example, it would appear that the lens protein is antigenically different from proteins of other tissues and organs (307). Antibodies are presumably not normally induced against it because it is restricted to the lens. Cellular antigens specifically concerned in the M–N and Rh blood groups appear to be restricted to erythrocytes. It may well be, therefore, that tumors are not antigenically different from the specific cells from which they arise. MacDowell and his associates (193, 194, 246) have shown that mice of an inbred line can be immunized against transplantable leukemia originating in the same line. But, curiously, such immunization does not protect against spontaneous leukemia. This and other aspects of immunity to mouse leukemia are discussed by MacDowell (193). Similar relations are known for other transplantable neoplastic cells (116, 120, 147). It is clear that more information about tissue- and organ-specific antibodies is needed before arguments along the above lines can be pushed further with profit.

If tumor cells differ from normal cells not genetically but in a manner analogous to that by which one type of normal cell differs from another, i.e., in some unknown but non-genic way, genes may still be important in their origin just as genes are important in determining the pathways of normal differentiation. An example of a gene that acts in this way is found in the genetically

recessive polymitotic character in Indian corn (12), in which the four products of sporogenesis fail to undergo the normal growth phase but immediately undergo a series of divisions in which, without multiplication, the ten chromosomes of the original cells are distributed among the daughter cells. This process continues until the supply of chromosomes is exhausted. These unregulated cells are not cancerous because their division potentialities are not unlimited. But they do indicate a principle that could well be concerned in tumor cell differentiation. Furthermore, genetic control of mutation rates may be differentially effective for various tissues (71).

It has been indicated that transplantable tumor cells may undergo spontaneous changes in transplantability, i.e., they may become less specific as to their host requirements. Since this specificity is apparently antigenically and genetically determined, the changes in specificity almost certainly represent gene mutations in the cancer cells. Although there need be no direct relation between mutations of this type and those that result in malignancy in the first place—assuming such mutations to occur—there is a parallel in that in both cases the organism serves as an enrichment culture for those cells with the highest growth rate. Looked at in this way and assuming the somatic mutation theory to be correct, the remarkable thing is not that malignancy changes are as frequent as they are but rather that they are not more frequent.

While the somatic mutation theory of cancer savors somewhat too strongly of fatalism for maximum comfort, it nevertheless cannot be disregarded. And ways of demonstrating its truth or falsity may yet be found.

As is well known, there are a number of tumors in which transmission can be accomplished in experimental animals by cell-free filtrates. These are definitely related to genes insofar as susceptibility to the filterable agent or virus is dependent on genetic constitution. If genes and viruses have as much in common as is thought by some, the relation may be much more direct. In fact, it appears possible that the virus and somatic mutation theories of cancer may not be mutually exclusive in any fundamental way.

N. Biosynthetic processes in Neurospora

If there does exist a one-to-one relation between genes and specific reactions, it should be possible to select from a series of induced gene mutations those concerned with particular reactions. Beadle and Tatum (19) have devised an experimental procedure for doing this with the fungus *Neurospora*. This organism was chosen because it can readily be grown in pure culture on a chemically defined medium, and because its life cycle is conveniently short and otherwise particularly favorable for genetic studies (178, 266).

This organism, commonly known as red bread mold, is heterothallic, that is, exists in two morphologically identical but physiologically different sexes or mating types. Each of these is haploid (seven chromosomes (192)) and by itself reproduces only vegetatively by mycelial growth or through the mediation of asexual spores. The hyphae of the growing mycelium are multinucleate. If mycelia of the two sex types are grown together on a suitable medium, hyphal

fusions occur and fruiting bodies containing sexual spores develop. These spores are produced in sac-like structures known as asci, and their development to maturity at 25°C. requires about 12 days from the time of fusion. The ascospores normally occur in sets of eight, one set per ascus, and they are arranged in a linear fashion like eight eggs in a narrow stocking.

The eight spores of a single ascus arise as a result of three nuclear divisions of an original zygote nucleus, i.e., the diploid nucleus resulting from fusion of haploid nuclei from the two parental strains. The first two of these divisions are meiotic, i.e., reduce the chromosomes from the diploid to the haploid condition. The third division is mitotic and simply divides each of the four meiotic products equationally, giving pairs of spores the members of each of which are genetically identical. The geometrical relations are such that if the parents differ in the alleles of a single gene, the eight ascospores of an ascus are of two kinds, four like one parent and four like the other. Their arrangement may be in two groups of four, one group like each parent, or in alternating pairs of the two parental types, depending on whether or not a crossover occurs between the segregating gene pair and the centromere. The relative frequencies of the two arrangements is a function of the distance of the segregating gene from the centromere. (See Lindegren (178) for further details.)

The nutritional requirements of *Neurospora* consist of (*1*) a carbon source (any of a number of sugars, starch, fat, etc.), (*2*) a nitrogen source (NO_3^-, NH_4^+, or any of several forms of organic nitrogen), (*3*) inorganic salts providing SO_4^{--}, PO_4^{---}, Ca^{++}, and K^+, a series of so-called trace elements (136), and (*4*) the B-group vitamin biotin. Satisfactory growth is obtained either in liquid or on a semi-solid (agar) medium if oxygen is supplied.

The method followed in obtaining biochemical mutants involves treating asexual spores with x-rays or ultraviolet light, making crosses with strains of the opposite sex, and then establishing single ascospore strains. On the basis of the life cycle indicated, such strains should be internally genetically homogeneous. They are grown on a medium as complete as possible in vitamins, amino acids, and other substances, the syntheses of which might be blocked as a result of gene mutations. Thus if such a strain no longer is able to synthesize thiamin, it can obtain this essential material from the medium. Loss of synthetic ability is detected by transferring asexual spores of the individual strains to a medium containing the minimal requirements of the wild-type strain. Failure to grow on this indicates a failure in some synthesis. For example, if vitamin B_1 were not made, growth would not occur on the minimal medium. Strains showing growth on "complete" but not on minimal medium are systematically tested on minimal medium with supplements of known compounds and in this way classified. Whether or not they differ genetically from the original strain is readily determined by making appropriate crosses, removing ascospores in order from the hybrid asci, and classifying the cultures from them for ability to grow on appropriate known media.

Strains, each differing from the original wild strain in a single relevant gene, have been obtained, each of which fails to grow in the absence of one of the

vitamins thiamin, pyridoxin, *p*-aminobenzoic acid, pantothenic acid, inositol, nicotinic acid, and choline (137). In each it is supposed that some one gene essential to the biosynthesis of the indicated vitamins has been inactivated or eliminated. In a similar way in another series of strains each requires a supplement of one of the known amino acids arginine, lysine, leucine, valine, methionine, tryptophan, proline, and threonine (74, 137). These, too, each differ from wild type by a single gene, the normal allele of which evidently plays some essential rôle in amino acid biosynthesis. In one case a mutant strain differing from wild type by a single gene requires both valine and isoleucine for normal growth (28). It is supposed that in the synthesis of these closely related amino acids there is a common reaction controlled by a given enzyme, the activity of which is in turn dependent on a specific gene.

Other strains require purines, pyrimidines, or the nucleosides or nucleotides of these for normal growth (137, 188, 310). Loring and Pierce (188) have reported that for a particular strain uridine and uridylic acid are many times more effective in promoting growth than uracil and that, while cytidine and cytidylic acid are active, cytosine is entirely inactive. These results suggest that the biosynthesis of cytidine and of cytidylic acid does not go through cytosine, a suggestion in line with the observation that free pyrimidines are not metabolized by mammals, whereas their ribonucleosides and nucleotides are (187).

An interesting practical application of mutant strains of *Neurospora* is in bioassays for particular vitamins and amino acids. So far, procedures have been developed for bioassays for pyridoxin (299, 300), *p*-aminobenzoic acid (312, 317), choline (136, 189a), inositol (15), and leucine (240, 255). An advantage of using mutant strains over naturally occurring organisms is that there is a greater freedom of choice as to the organism used and also as to its specificity.

Still other mutant strains have been obtained in which reduction of nitrate to nitrite is genetically blocked (137). Horowitz (137) has studied two strains, one unable to utilize any fatty acid as a carbon source and another able to use saturated but not unsaturated fatty acids.

It is clear from the kinds of mutants so far found that the original assumption that genes must control many if not all enzymatic reactions is essentially correct. Apparently all that is necessary is that the conditions of selection be properly determined, and mutations can be obtained in which almost any predetermined reaction is blocked. It will be recalled that this technique was followed by Moewus in determining the relations of genes to the synthesis of motility and sex hormones in *Chlamydamonas*.

One of the most completely understood series of genetically controlled reactions in *Neurospora* is that leading to the synthesis of the amino acid arginine. Srb and Horowitz (283) investigated fifteen mutant strains, each of which required arginine or a related compound for normal growth. One of these grows only if arginine itself is supplied. Two others, genetically different from each other, grow if either arginine or citrulline is supplied. Four other strains, genetically different from one another and from the first three, have their growth requirements met by arginine, citrulline, or ornithine. The remaining eight of

the fifteen mutants were found to represent duplicate or replicate occurrences of the same gene mutations as are concerned in the first seven mentioned.

The combined biochemical and genetic interpretation of arginine synthesis in *Neurospora* is indicated in figure 6. Apparently essentially the same mechanism operates here as in the mammalian liver, as determined by Krebs and Henseleit (159). There is an arginase found in *Neurospora* which splits arginine to ornithine and urea. With the intervention of urease, the urea is further degraded to carbon dioxide and ammonia. It is evident that the one-gene–one-reaction concept applies to this series of reactions, especially when it is recalled that the conversion of ornithine to citrulline is postulated on independent grounds to involve two steps.

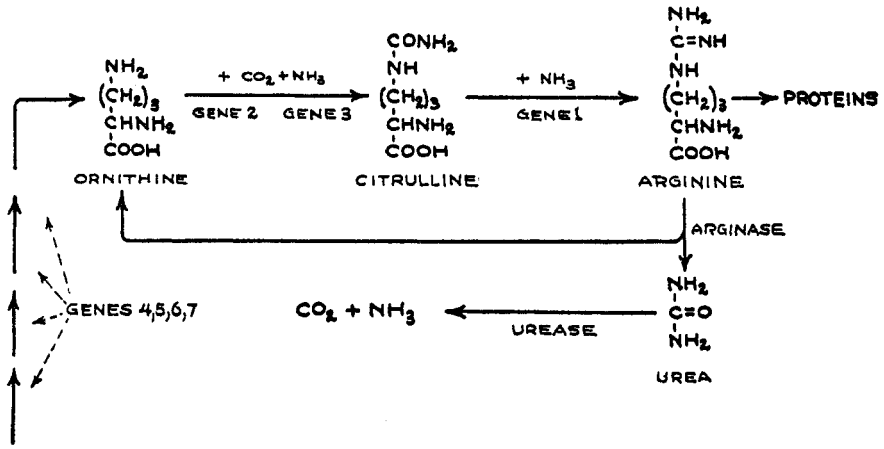


FIG. 6. Arginine cycle in *Neurospora* (after Srb and Horowitz (283))

Somewhat analogous relations are found in the synthesis of tryptophan in *Neurospora*. Here, however, the precursors were not so certainly known as in the case of arginine. Tatum and Bonner (313) have demonstrated that the final step in tryptophan synthesis involves the condensation of indole and serine, and that the serine in turn is made from *o*-aminobenzoic acid according to the reactions indicated in figure 7. A mutant strain is known which cannot convert anthranilic acid to indole. In the presence of a minimal amount of indole or tryptophan for growth, such a strain accumulates anthranilic acid and excretes it into the culture medium (314). In fact, it was this accumulation of the precursor of indole that led to its identification. Other mutant strains are known in which anthranilic acid is not synthesized. They are able to grow normally if either anthranilic acid, indole, or tryptophan is supplied.

In the case of indole formation from anthranilic acid it is clear how a genetic method can become a powerful tool in investigating metabolism. The inactivation of specific genes is equivalent to the chemical poisoning of specific enzymes, with the important difference that genes are highly specific, whereas enzyme poisons are often discouragingly non-specific. The genetic method is now being

applied in the study of several synthetic processes; for example, Horowitz (137) is studying two genetically distinct mutants that require choline or a related compound for normal growth. In the presence of a small amount of choline, one of these accumulates a choline precursor which it cannot itself use effectively but which the second strain can use quite readily, by converting it to choline. The first strain carries the synthesis up to substance X but cannot continue because of a defective gene which presumably results in a defective enzyme. In the second strain, this gene and the corresponding enzyme are active and the series of reactions can proceed normally from X to choline.

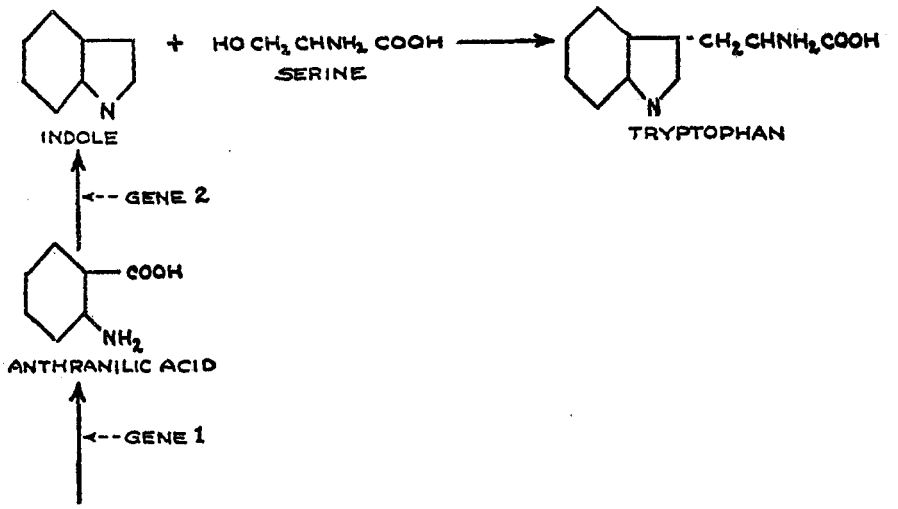


FIG. 7. Tryptophan synthesis in *Neurospora* (after Tatum, Bonner, and Beadle (314))

That different genes concerned with a series of reactions leading to a particular end product control different steps in the process is illustrated in fusion strains in which a given mycelium contains a mixture of two or more genetic types of nuclei. If two genetically different strains, each of which requires nicotinic acid or a related compound for growth, and both of the same sex, are placed together on an agar medium in which no nicotinic acid is supplied, hyphal fusion occurs, nuclei from the two strands become intermingled in a common cytoplasm, and normal growth is resumed. This complementary action, or intracellular internuclear symbiosis, shows that each of the two mutant genes is recessive. Each type of nucleus carries a normal allele of the mutant gene in the other. One carries the synthesis up to X; the other carries on from X to the final product. Several such instances have been studied by Beadle and Coonradt (17). The net result is similar to that in interspecific symbiosis, in which each species supplies a growth factor lacking in the other (310). Such coöperation in carrying out syntheses may well be a factor in hybrid vigor and in the evolutionary origin of sexual reproduction (17).

O. Miscellaneous specific reactions

Winge and Laustsen (340) have studied the inheritance of ability to ferment specific sugars in yeast. They find that in crosses in which there is a difference in this respect, diploid hybrids have the ability to ferment, i.e., ability to ferment a given sugar is dominant over inability. This was shown to be true for sucrose, melibiose, and raffinose. Following reduction divisions in which haploid ascospores are formed, segregations were observed. The material as used by these authors, however, was not favorable for establishing whether or not precise ratios were obtained. It is nevertheless probable, as Winge and Laustsen conclude, that specific genes control the production of enzymes which hydrolyze or phosphorolyze the sugars mentioned. More recently Lindegren, Spiegelman, and Lindegren (179) have reinvestigated the inheritance of ability to ferment melibiose in hybrids between *Saccharomyces cerevisiae* and *S. carlsbergensis*, and have found that in diploid strains ability to ferment is dominant over inability and that in the formation of ascospores regular segregations occur. *S. carlsbergensis*, however, was found to differ from *S. cerevisiae* in carrying dominant alleles of two genes. The active allele of either gene conditions the presence of an enzyme catalyzing the splitting of the disaccharide. It is implied by Lindegren *et al.* that only *one* enzyme is concerned, an interpretation at variance with the one-gene–one-enzyme concept, at least in its simpler form. An alternative view, which certainly has not been excluded and which is in agreement with most recent views on the splitting of compound sugars, is that one gene concerns the production of a hydrolytic enzyme, while the other is responsible for the specificity of an enzyme catalyzing phosphorolytic splitting of the sugar. On this basis *S. cerevisiae* would have inactive recessive alleles of both these genes.

An additional finding of Lindegren *et al.* and Spiegelman *et al.* that is of the greatest significance is that if one enzyme (possibly either one but probably a particular one) is present in the cytoplasm of an ascospore which does not carry the active allele of either gene, and if the spore and its descendents are grown in the continued presence of melibiose, the strain continues to split the sugar indefinitely (179, 281a). The relation of this observation to theories of enzyme reproduction and to mechanisms of gene action will be discussed later (page 86).

The production of the enzyme amylase in the silkworm has been reported by Matsumura to be genetically controlled (original paper unavailable to author, cited from Wright (347)). Some strains show a strong amylase activity, whereas in others it is weak. It is reported that one gene pair differentiates strong from weak races with respect to the digestive juices while a second gene, strongly linked with the first, differentiates between strong and weak races as far as the body fluid enzyme is concerned. Whether or not the two enzymes are in any way different is apparently not known. Again it seems possible that one might be a phosphorylase and the other a hydrolase.

A type of Indian corn known as "waxy" has been known for many years as a curiosity and as a useful genetic character. Waxy corn kernels have red-stain-

ing rather than normal blue-staining starch (33). This is also true of the embryo sac and pollen grain starch. In the latter the difference in starch in the two kinds of haploid pollen grains produced by heterozygous plants becomes evident after only one or two nuclear divisions of the meiotic products and after only a few days. Brink and others (33) have studied the chemical differences between waxy and normal tissues and find a difference in amylase activity. More recently it has been found that there are important differences in the physical properties of waxy and normal starch which suggest that the waxy type of starch contains a higher proportion of branched-chain molecules than does normal starch (132, 282). Here again the two alleles of a specific gene appear to impart different specificities to the enzymes elaborated under their guidance.

In white clover (*Trifoleum repens*), as well as in other leguminous plants, there are inter-plant differences in cyanogenetic glucoside content. These compounds may liberate hydrogen cyanide under certain conditions and therefore be toxic to livestock. The glucosides concerned in white clover are lotaustralin and linamarin (207). Under the influence of the enzyme linamerase (56) these are split to hydrogen cyanide, glucose, and a ketone (ethyl methyl ketone and acetone, respectively). It has been shown that strains may differ genetically both in their ability to synthesize the cyanogenetic glucosides and in the presence of the hydrolytic enzyme (4, 57, 58, 337). Each is dependent on the dominant allele of a specific gene, and the two genes are inherited independently. Plants carrying the normal allele for glucoside synthesis but not that necessary for the presence of the hydrolytic enzyme have the glucoside present. Atwood and Sullivan (4) raise the question of how the glucoside can be present in the absence of the enzyme which they evidently believe should be concerned in synthesis as well as degradation. They propose that the recessive allele of the enzyme gene is not completely inactive and that therefore plants homozygous for it have sufficient enzyme to carry out the synthesis. An alternative and preferable view is that the enzyme responsible for hydrolysis is not concerned in synthesis.

A mendelian recessive trait is known in the rabbit in which the fat is yellow rather than white, as in normal rabbits (42, 230). This apparently is the result of inability to oxidize ingested carotenoid pigments which are then accumulated in the fat (338). If homozygous yellow-fatted animals are fed on a carotenoid-free diet, they have white fat. The dominant allele of the gene concerned is apparently in control of a specific enzyme which has been referred to as xanthophyllase (42).

Zechmeister and his coworkers (354) have shown that the "tangerine" and normal red tomato, known to differ in one pigment gene, contain stereoisomers of lycopene. The tangerine type contains a *cis*-isomer known as prolycopene, while the red form contains all *trans*-lycopene. In the biosynthesis of lycopene the prolycopene isomer may be an intermediate or it may be a secondary product resulting from a genetically blocked reaction.

Levy and Michel (175) observed that individual rabbits differ in the ability of their blood to hydrolyze the plant alkaloid atropine. It is known that this

reaction is enzymatically catalyzed. Sawin and Glick (259) have demonstrated that the enzyme concerned, atropinesterase, is dependent for its presence on the dominant allele of a specific gene. Ability to hydrolyze atropine is therefore inherited as a simple mendelian dominant trait.

P. Summary of specific gene-controlled reactions

A summary of the specific chemical reactions known to be gene-controlled in at least one organism is given in table 1. Cases such as those of oxidation and of xanthophyll in the rabbit and of 3,4-dihydroxyphenylalanine to melanin in various vertebrates are omitted because the products of the reactions are not known.

It is an interesting commentary on the development of biochemical genetics that twenty-four of the twenty-five reactions listed here have been related to specific genes within the last ten years, while three-fourths of them have been so related during the last five or six years.

V. CHEMICAL NATURE OF CHROMOSOMES AND GENES

During the past several years rapid advances have been made in our knowledge of the chemical and physical properties of chromatin. The literature has become substantial, but on many important points no general agreement has yet been reached. Because of this it is possible in the present review only to indicate briefly some of the directions along which progress is being made. For detailed treatment of the subject reference is made to recent reviews by Mirsky (211), Gulick (123, 124), Schultz (263, 264), and Muller (219), and particularly Volume 9 of the Cold Spring Harbor Symposia on Quantitative Biology—*Genes and Chromosomes; Structure and Organization*, which reviews pertinent work from several viewpoints.

A. Isolation and analysis of chromatin

An obvious and direct method of determining what the hereditary material is chemically is to isolate nuclei or chromatin which contain the genes and subject them to analysis. Unfortunately this is not simple, for it is certain that chromatin contains many kinds of genes and it is most probable that in addition to genes it contains much non-genic material. Furthermore, the compounds involved are not resistant to rough chemical treatment and the very properties in which we are most interested may be destroyed in the process of isolating them. Miescher, who worked near the end of the last century, showed that sperm, which are made up largely of nuclei with little cytoplasm, and pus cell nuclei contain proteins and nucleic acid. Similar methods were used later by Kossell and by Levene. It is now known that the methods used by all of these early workers were much too drastic. They employed acids, alkalis, and heat and certainly greatly altered the physical properties of the materials with which they were working (211).

In 1924 Hammarsten began a series of studies on nucleic acid isolated from cell nuclei by simple extraction in large volumes of water. Material isolated

TABLE 1

Summary of specific reactions known to be gene controlled

In all cases, where it can be determined, the ability of the organism to carry out the reaction is inherited as a dominant trait

SYSTEM OF REACTIONS	REACTION	ORGANISM	AUTHORITY	REMARKS
Anthocyanins and related pigments....	Cyanidin → pelargonidin	*Callistephis*	Wit (341)	
		Streptocarpus	Lawrence *et al.* (172)	
		Lathyrus	Beale *et al.* (21)	
	Cyanidin → delphinidin	*Callistephis*	Wit (341)	
		Streptocarpus	Lawrence *et al.* (172)	
		Lathyrus	Beale *et al.* (21)	
	Anthocyaninidin-3-glycoside → 3,5-glycoside	*Verbena*	Lawrence and Price (170)	
	Quercetin-3-glucoside → cyanidin-3-glucoside	*Zea*	Sando *et al.* (257)	
Tyrosine metabolism.....	2,5-Dihydroxyphenylacetic acid → acetoacetic acid	Man	Garrod (101)	
	Phenylpyruvic acid → *p*-hydroxyphenylpyruvic acid	Man	Fölling (98)	
Tryptophan metabolism..	Tryptophan → α-oxytryptophan	Insects	Butenandt *et al.* (37)	
	o-Aminobenzoic acid → indole	*Neurospora*	Tatum *et al.* (314)	
Arginine synthesis.....	Ornithine → citrulline	*Neurospora*	Srb and Horowitz (283)	
	Citrulline → arginine	*Neurospora*	Srb and Horowitz (283)	
Purine metabolism.....	Uric acid → allantoin.....	Dog	Trimble and Keeler (321)	
Thiamin.....	Thiazole + pyrimidine → thiamin	*Neurospora*	Tatum and Beadle (311)	
Pantothenic acid.....	Pantoyl lactone + β-alanine → pantothenic acid	*Neurospora*	Tatum (310)	

TABLE 1—*Continued*

SYSTEM OF REACTIONS	REACTION	ORGANISM	AUTHORITY	REMARKS
Carotenoid pigments.....	Protocrocin → crocin + gynotermone	*Chlamydamonas*	Moewus (214)	
	Gynotermone → androtermone	*Chlamydamonas*	Moewus (214)	
	cis-Cronin → *cis*-dimethyl crocetin	*Chlamydamonas*	Moewus (214)	
	trans-Crocin → *trans*-dimethylcrocetin	*Chlamydamonas*	Moewus (214)	Enzyme active only in light
	Prolycopene (*cis*) → lycopene (*trans*)	Tomato	Zechmeister *et al.* (352)	
Carbohydrate splitting*....	Sucrose → fructose + glucose	Yeast	Winge and Laustsen (340)	Enzyme inferred
	Melibiose → glucose + galactose	Yeast	Winge and Laustsen (340)	Enzyme inferred
	Raffinose → fructose + melibiose	Yeast	Winge and Laustsen (340) Lindegren *et al.* (179)	Enzyme inferred
Cyanogenetic glucoside hydrolysis.....	Lotaustralin → ethyl methyl ketone + HCN + glucose	Clover	Corkill (58) Atwood and Sanford (4)	Enzyme *in vitro*
	Linamarin → acetone + HCN + glucose	Clover	Corkill (58) Atwood and Sanford (4)	Enzyme *in vitro*
Atropine hydrolysis.....	Atropine → tropine + tropic acid	Rabbit	Sawin and Glick (259)	Enzyme *in vitro*
Nitrate reduction.....	Nitrate → nitrite	*Neurospora*	Horowitz *et al.* (137)	

* Not determined for certain whether reaction is hydrolysis or phosphorolysis.

in this way was studied by several investigators (review, 211) and shown to be a highly polymerized fibrous material, the particles ranging from a molecular weight of 200,000 to over 1,000,000. This nucleic acid is known to contain the pentose desoxyribose and is commonly known as desoxyribonucleic acid. X-ray

diffraction analysis shows that desoxyribonucleic acid fibers have strong periods along their longitudinal axes at intervals of 3.34 Å. This spacing is almost exactly the same as that of the amino acid residues in a fully extended polypeptide, a fact of significance in the union of nucleic acids and proteins through salt and other linkages. The purine and pyrimidine nucleotides (base plus sugar plus phosphoric acid) are assumed to lie in planes perpendicular to the fiber axis.

Mirsky and Pollister (212, 213) have recently developed a method of isolating intact nucleoproteins from nuclei. The nuclei are first separated from the remainders of the cells in any of several ways (review, 211). In isolating the nucleoproteins, the chromatin threads themselves may first be isolated (213), or the extraction may be made from intact nuclei. The procedure is dependent on the fact that the nucleoproteins of the nucleus are soluble in 1.0 *M* neutral sodium chloride solution. They are precipitated in neutral physiological saline (0.14 *M* sodium chloride for birds and mammals) as a strongly fibrous material, showing birefringence of flow. So far as can be determined, the nucleic acid component of nuclear nucleoproteins is the same from different organisms, although the criteria by which this is determined are limited. The protein component, however, may be more than 90 per cent protamine from certain fish sperm, or almost entirely histone from other nuclei. Curiously, the protein from salmon sperm appears to be largely protamine, while that from the erythrocyte nuclei of the same organism is largely histone (211). Mirsky and Pollister have made the significant observation that their preparations of histone contain no tryptophan, while those of protamines do. Protamines, in contrast to histones, do not contain tyrosine (211).

The nucleic acid of nucleohistones and nucleoprotamines is rather easily separated by treatment with strong salt solution or by dialysis. However, the nucleoproteins migrate as single units in an electric field.

That chromosomes are composed mainly if not entirely of histone or protamine nucleoproteins as argued by Mirsky and Pollister is denied by others. Mayer and Gulick (202), for example, claim to have isolated from veal thymus gland nuclei a sulfur-rich protein and a globulin in addition to nucleohistone. Stedmann and Stedmann (292) have gone even further in claiming that the characteristic protein of chromatin is neither protamin nor histone but a newly recognized protein rich in both basic and dicarboxylic amino acids which they call chromosomin.

In summary it seems fair to say that the direct chemical attack tells us that chromosomes contain substantial amounts of nucleoprotein made up of a highly polymerized desoxyribonucleic acid and either a protamine or a histone. The relation between protamine and the histones which apparently are found in the nuclei of different cells of the same animal is not entirely clear. Nor is it certain what other types of protein, if any, are present in chromosomes, what their relative quantities are, and whether or not they are combined with nucleic acid. On the basis of the observation that apparently *either* protamine or histone alone can be present in chromatin, it seems improbable that either is responsible for the characteristic properties of genes, although Mirsky and

Pollister (213) are inclined to believe that histones are capable of playing this important rôle.

B. Physical properties of chromosomes

It is possible to observe chromosomes in the living cell and even to manipulate them with microneedles. In this way, for example, salivary gland chromosomes of *Diptera* are seen to exhibit a characteristic stickiness, to show reversible swelling and shrinking in response to changes in osmotic concentration of the medium in which they are observed, and to be capable of being reversibly stretched to double their normal lengths (34, 35).

Attempts have been made to study chromosome structure by means of the x-ray diffraction methods so successfully used in the study of various protein fibers by Astbury and others, but technical difficulties have so far prevented definite conclusions from being arrived at in this way (35).

C. Staining reactions

The classical methods of studying chromosomes under the microscope have involved the use of basic dyes. While these, especially as applied to the giant salivary gland chromosomes of the *Diptera*, have contributed much to our understanding of the behavior of chromosomes during cell division, they are in general not sufficiently specific to lead to definite conclusions regarding chemical composition.

A significant advance was made in 1924, when Feulgen and Rossenbeck (96) developed the so-called Feulgen reaction as a specific test for the nucleic acid of the nucleus. This reaction was shown later by Levene to be specific for desoxyribose. Since this pentose appears not to occur except as a component of nucleic acid, the Feulgen reaction is specific for desoxyribonucleic acid. The related ribonucleic acid, found in the nucleolus and in the cytoplasm, does not give the reaction. Desoxyribonucleic acid was soon shown to be present in the cell nuclei of all organisms, both plant and animal (211), and even in bacteria (153, 247), which by many had been thought not to contain chromatin. The desoxyribonucleic acid is confined to the chromosomes of the nuclei of higher organisms and to what appear to be homologous bodies in bacteria. In the giant salivary gland chromosomes the nucleic acid is largely confined to definite transverse bands.

It is unfortunate that no reaction is known that is as specific for ribonucleic acid. Because of this, less direct methods of detecting this analogous compound must be resorted to and in the presence of large amounts of desoxyribonucleic acid this becomes technically most difficult.

Recently Calvin, Kidani, and Goldschmidt (39a) have applied various reagents to salivary gland chromosomes in an attempt to gain information on their structure. While the results of these attempts are most fascinating, they have not been interpreted in a manner acceptable to many cytologists (e.g., Painter (227)), and accordingly, the interested reader is referred to the original papers.

D. Ultraviolet-absorption methods

Following earlier work of Kohler, Lucas, and Stark, and others (review, 211) in which the quartz microscope was used for photographing chromosomes with ultraviolet light, Caspersson (41) developed extremely sensitive methods for determining nucleic acid distribution in cells. These methods depend on the fact that the pyrimidine and purine rings of nucleic acid contribute to a characteristic absorption in the ultraviolet with a strong maximum at 2600 Å. They do not distinguish between the ribose and desoxyribose types. Apparently the purines and pyrimidines are largely confined to nucleic acid in most cells, although it is well known that the striated muscle cell is a marked exception in this regard. Since the ultraviolet-absorption technique is most useful in conjunction with both the Feulgen reaction and enzyme digestion, a discussion of conclusions supported by it is deferred until the enzyme digestion method is presented.

E. Enzyme digestion

Theoretically it should be possible to determine what materials go to make up chromosomes by treating these bodies with specific enzymes. This was appreciated by Caspersson, and he combined this method with that of ultraviolet absorption. For example, in studying the salivary gland chromosomes, ultraviolet absorption indicates accumulation of nucleic acid in the transverse bands that stain dark with basic dyes. If the protein component of the chromosome is digested with trypsin and the nucleic acid precipitated with lanthanum so as to remain in place, the bands now composed of nucleic acid only remain. Mazia and Jaeger (203), Schultz (264), Frolova (100), and others (211) have extended this method, making use of trypsin, pepsin, papain, phosphatase, nucleases, and preparations of other enzymes. The continuous framework of the salivary gland chromosome seems to be a protein, possibly of the histone type. Digestion with trypsin destroys the continuity, while pepsin, which does not break histone down completely, does not (41, 204). The continuous structure remains after removal of nucleic acid by nuclease digestion according to Mazia (204), and can be stained with ninhydrin, but Schultz (264) reports that under certain conditions salivary gland chromosomes are almost completely digested by crystalline ribonuclease.

There are several possible obvious sources of error in using the enzyme digestion method in studying chromosome structure. Some of these have been recognized but others apparently have not, at least by certain investigators. A most serious difficulty is that of being sure an enzyme preparation is pure. For example, crude nuclease preparations so often used evidently contain several known enzymes and possibly others not known (204).

F. Conclusions

It appears from the evidence obtained by the various methods indicated that chromosomes are made up of a protein framework along which nucleic acid is combined at intervals, possibly by salt-like linkages. The nature of the protein

framework is not known. It is supposed by some to be histone, but this view raises the question of what constitutes the framework in those chromosomes containing protamine rather than histone (211). Possibly other proteins are present which provide for the longitudinal continuity.

The cumulative evidence indicates nucleoprotein as the principal component of genes. Recent work on the chemical nature of viruses, which have several important properties in common with genes, lends support to this inference (page 83). The protein component may be histone or some other protein. It is probable that gene specificity is determined by the protein component. There is, however, recent evidence from work on the nucleic acid of pneumococci suggesting that this component may possibly play a part in determining the specificities of individual genes (6).

The part played by nucleic acid in gene and chromosome duplication remains a mystery, in spite of the fact that definite cycles involving this component are known to be correlated with cell division and other phenomena of genetic importance. During prophase there is a strong increase in desoxyribonucleic acid in the nucleus and a concomitant decrease in ribonucleic acid in the cytoplasm. This phase of the cycle is completed at metaphase, when the chromosomes are heavily charged with desoxyribonucleic acid and the cytoplasm almost devoid of the ribose analogue. During anaphase and telophase the cycle is reversed, with a maximum of ribonucleic acid in the cytoplasm and a minimum amount of its desoxyribose relative in the nucleus at the resting stage (review, 227). The nucleolus, a spherical body within the nucleus containing ribonucleic acid, shows a negative correlation with the chromosomes in nucleic acid content. During the resting stage it is heavily charged with ribose-type nucleic acid but disappears during active division (211). It appears from these and other observations (227) that ribonucleic acid acts as a storage form which can be drawn upon during cell division or at other times of active protein synthesis. How the transformation from one type to the other is accomplished chemically is not known, however.

Certain regions of the chromosomes in most species are "heterochromatic." These regions are heavily charged with desoxyribonucleic acid at certain stages and are consequently stained heavily with basic dyes. They have been assumed to be genetically "inert," in the sense that ordinary genes are not found to be carried in them, but recently Mather (201) has suggested that they may be important in carrying "polygenes" (see page 80). Evidently they have some important part to play in desoxyribonucleic acid economy but precisely what this is is not known (reviews: 263, 264).

VI. GENE MUTATION

A. Spontaneous changes

The frequency with which genes undergo spontaneous mutation varies both with the organism and with particular genes within one organism. Muller has pointed out that the rate at which lethal mutations arise in the X chromo-

some of *Drosophila* per unit time is sufficient to produce lethals in a high proportion of the X chromosomes in man during the course of one generation. Obviously no such rate occurs. Evolutionary flexibility demands that the rate be appreciable, but natural selection under fixed environmental conditions tends to hold it down. The equilibrium will be expected to be reached on the basis of generations as units rather than absolute time. Assuming that genes vary chemically, they must vary in their stabilities and hence be differentially subject to change through inevitable changes in their surroundings.

The temperature coefficient for lethal mutations in *Drosophila* has been estimated for temperatures from 8° to 31°C. Because of the low rates the measurements are subject to a large error, but they indicate a temperature coefficient of about 5 (review, 234). Temperature shocks (treatments for short times at temperatures below or above those at which the organism is capable of develop ing normally) appear to increase mutation rates significantly (234).

As has already been mentioned (page 22) genes are known which influence the spontaneous mutability of other genes. These may be general in their effects (14, 199, 234, 306), or they may specifically affect certain other genes (242). It is presumably on the variability resulting from such mutation-influencing gene changes that natural selection acts to keep the over-all mutation rate at a favorable level.

For most individual genes the spontaneous rate of mutation is so low as to make it almost impossible to measure experimentally except in particularly favorable cases. Haldane (128) has calculated that the normal allele of the gene controlling hemophilia in man must mutate to a defective allele approximately once per 50,000 X chromosomes in each generation. For various endosperm characters in maize in which large numbers can be obtained readily, Stadler (284, 285) has observed mutation rates for various genes ranging from one or less to five hundred per million chromosomes per generation. In the case of the so-called mutable or unstable genes, of which several examples are known (71), the mutation rate is much higher. Often thousands of mutant changes occur in a single organism during the course of its development.

In connection with spontaneous mutation rates it should be pointed out that these are usually from the normal allele to an allele that can be regarded as inactive or defective in some way. Actually it is experimentally almost impossible to tell, except by waiting for back-mutation to occur, whether a mutation that inactivates is a true gene change, with the gene retaining its power of self-duplication, or a complete physical loss (191, 284).

B. Induced changes

While there can be no doubt that the discovery by Muller in 1927 that mutations in *Drosophila* can be increased many fold by treatment with x-rays represents one of the outstanding achievements of genetics, it is nevertheless true that we still cannot say precisely how the effect is brought about. X-rays, gamma rays, neutrons, and ultraviolet radiation are all capable of delivering energy to the gene in a manner capable of causing mutations. X-rays and

gamma rays produce their effects through the mediation of fast electrons from atoms in the tissue. These produce ions as they pass through cells. In neutron treatment protons have a similar effect, except that because of their greater mass the ionizations along the particle path are denser than in the case of electrons. In the absorption of a quantum of ultraviolet radiation by a cell, less energy is involved than in an ionization and it seems quite clear that the effect of this type of radiation is different in other respects from that of ionizing particles.

Within the x-ray spectrum, gene mutations are apparently strictly proportional to dosage and are independent of wave length and intensity (reviews: 68, 94, 218, 319). This means that single ionizations must be responsible for the change, for otherwise there would have to be wave-length and intensity effects. On the other hand, it is not certain whether the effective ionization has to occur in precisely the molecule or group of molecules in which the final effect is brought about or whether it is possible for the necessary energy to be transferred from the site of absorption to the locus of action (reviews: 94, 99, 218, 319).

The magnitude of the effect of radiation may be great. Muller (218) points out that with a treatment of 10,000 r-units the frequency of lethals in the X chromosome of *Drosophila* is increased about 100-fold over that occurring spontaneously in one generation of 10 days. Since this treatment may be applied in a period of an hour or less, the increase per unit time may be as high as 35,000-fold. As with spontaneous mutations, most mutant changes induced by x-rays are lethal, i.e., the organism cannot develop when homozygous for them. An important question concerns whether the proportions of types of mutant changes is the same with x-ray treatment as those found naturally. If gross chromosome aberrations such as translocations (mutual exchange of noncorresponding chromosome segments) and inversions (reversal of gene sequence in a chromosome segment) are included, it is clear that the proportion of types is not the same as that for spontaneous mutations, for x-rays produce a relatively greater increase in chromosome aberrations than in gene mutations.

Many x-ray lethals are found on cytological examination to be small deficiencies (small segments of a chromosome removed with the broken ends rejoined). This fact raises the question of whether the sole effect of ionizing radiation is to remove genes completely (284, 285). Small deficiencies are known to produce phenotypic results similar to if not identical with gene changes which supposedly do not involve loss (191). In *Drosophila* the answer seems to be clear that at least some x-ray mutations are not losses, because "back-mutations" can be induced (319). In maize, on the other hand, the answer is not so clear. In a special test, Stadler (285) found no x-ray mutants of *A* to *a* that gave fully viable plants in the homozygous state. Furthermore, he was unable to induce mutation from *a* to *A*, using an *a* allele known to be subject to back-mutation in the presence of the *Dt* gene (page 22), in a population sufficient to give almost a million *A* losses under similar treatment (286).

Ultraviolet, on the other hand, clearly gives rise to mutations in maize similar

to those that occur spontaneously. Many *A* to *a* mutants induced in this way appear to be fully viable in the homozygous state (285).

Unlike x-rays, ultraviolet shows a strong wave-length effect in producing mutations. The curve of effectiveness in producing mutations against the wave length is sufficiently similar to the absorption spectrum of nucleic acid over the same wave lengths as to support strongly the view that this substance is closely associated with genes if not indeed a component of them (134, 135, 152, 258, 289).

Unlike gene mutations, gross chromosome aberrations do not show a linear increase in frequency with increasing dosage of x-rays, and their frequency is little if at all increased by ultraviolet treatment. The situation appears to be that these changes (translocations, inversions, and long deficiencies) require the simultaneous occurrence of two breaks in the chromosome.

If there were no complicating factors, the frequency of these would be expected to go up as the square of the dose (218). It seems, however, that these breaks do not remain available for rearrangements longer than a certain time. In *Drosophila* sperm this appears to be until they fertilize the egg, and therefore no time–intensity factor need be considered when sperm are treated (218). In *Tradescantia* (spiderwort) microspores, on the other hand, breaks heal within a matter of minutes (173, 260, 261) and complications are introduced unless time of treatment is kept constant in experiments in which dosage is varied.

In the production of chromosome breaks such as are concerned in gross chromosome rearrangements, apparently several ionizations are required for a single break (108, 173). Lea and Catcheside (173) have recently calculated that something of the order of seventeen ionizations induced in a chromatid (one unit of a divided but not yet separated chromosome) as an ionizing particle traverses it (diameter ca. 0.1μ) are required to break it. This leads to the prediction that x-rays of different wave lengths will vary in their effectiveness in inducing breaks. The fact that experimental tests show excellent quantitative agreement with predictions (44) lends strong support to their arguments.

Swanson (309) has shown in a striking manner that the ultraviolet effect is different from that of ionizing particles. If x-ray treatments are preceded by ultraviolet treatment, the x-ray effects on chromosome aberrations are partially suppressed. According to Swanson, the ultraviolet probably produces a physical change in the chromosome matrix of sheath of such a nature that restitution of breaks is favored over rearrangements of broken ends.

Since it is almost certain that gene mutations involve chemical changes of some kind in the gene, it would seem most probable that these changes could be induced at will by the proper chemical treatment. Attempts to accomplish this have been many, but almost all of them have been disappointing. It is true that many reports of success have appeared in the literature but, except for those of a special type to be mentioned below, none has been confirmed. *Drosophila* eggs have been soaked in solutions of various chemicals, chemicals have been injected into young *Drosophila* larvae, and many other treatments have been tried. Some give slight effects of doubtful statistical significance

(review, 169), while others such as feeding heavy water (353), proteolytic enzymes (352), and nucleic acid (107, 218) seem to have no effect whatever. Steinberg and Thom (293) have reported positive results with nitrite treatments in *Penicillium*, but the alleged mutations cannot be demonstrated to be due to gene changes because this organism has no perfect stage. Their results have not been confirmed in more favorable organisms. Steir and Castor (298) report a permanent change in yeast cells following cyanide treatment, but no evidence has been presented that a gene change is involved. Recently Auerbach and Robson (5) have reported that allyl isothiocyanate (mustard oil) induces mutations in *Drosophila*. Further reports on this will be awaited with interest.

As a special category, the striking effects of the alkaloid colchicine on cell division should be mentioned (72). This drug has the curious property of inhibiting cytoplasmic division by interfering with the spindle mechanism while allowing the chromosomes to reproduce normally. The result is that chromosomes become multiplied in treated cells. This is a most useful tool in both theoretical and applied genetics, but there is no evidence that any change in the genes is produced by it.

The difficulty of chemically inducing gene changes is undoubtedly in part due to the difficulty of getting reagents to the gene without killing the cell in which they are carried. It is a well-known fact that the nucleus is remarkably resistant to staining by vital dyes, an indication of its general resistance to the entrance of foreign materials. It is nonetheless a reasonable hope that someone will some day discover the right trick to bring about transmutation of the gene with specific chemical treatment.

More than twenty years ago Guyer and Smith (125, 126) reported the induction of heritable changes in the rabbit by injecting anti-rabbit-lens sera into pregnant rabbits. The offspring showed eye defects which were transmitted. Several attempts to confirm this result by other workers failed, and the antibody-induced mutations were explained away by geneticists. Recently, however, Hyde (307) has repeated the Guyer and Smith experiment on an extensive scale with adequate controls and has found results essentially identical with those of the earlier workers. In interpreting this work, Sturtevant (307) suggests that, since it is probable that genes and antigens have physically corresponding specificities, it is possible that genes, like antigens, can combine with antibodies of corresponding specificities. If this were to occur, it seems possible that gene duplication would be interfered with in such a way that daughter genes would be absent or modified, i.e., mutated. Emerson (85) has attempted to test this possibility with *Neurospora*. Extracellular adaptive enzymes were obtained from culture filtrates, immune sera directed against these were produced in rabbits, and *Neurospora* cells then treated with the antibody-containing sera. Here enzymes replace the lens protein as antigens. Although the technical difficulties of obtaining known enzymes in pure form are many and have not yet been overcome, preliminary tests indicate some hope that immunological specificities may be made use of to "direct" mutations.

The transformation of types in *Pneumococcus* is a case in which directed mu-

tation seems to have been accomplished. It has been known for some time that if avirulent rough (no polysaccharide capsule) mutant forms of one type are allowed to back-mutate to a virulent smooth form in the presence of an extract from a virulent smooth form of another type, the back-mutation is associated with a change of type to that of the form supplying the type-directing substance. In the absence of such a directing agent the reversion of rough to smooth is invariably brought about with no change in type; that is, the rough reverts to a smooth of the same type as the smooth from which the rough was originally obtained. Avery, MacLeod, and McCarty (6) have recently isolated the directing agent in pure form and found it to be a nucleic acid. By every available test the activity of this is due to nucleic acid and not to some impurity present in undetectably small amounts. The activity is very high and a small amount of this specific nucleic acid causes the reverted type-changed strain to make more type-specific nucleic acid like that supplied. This may mean that a specific gene has been mutated, or that the type-specific nucleic acid is capable of autocatalytic reproduction. The latter alternative amounts to essentially the same thing as the suggestion of Wright (349) that the nucleic acid itself may be the gene and that the transformation of type is brought about not by mutation but instead by actual transfer of the gene.

Unfortunately, pneumococci do not reproduce sexually, so no direct test can be made to determine the relation of type-change alterations to gene mutations as known in other organisms. This difficulty applies as well to bacterial variations induced in other ways (70, 119, 250a).

VII. GENE ACTION

A. Interaction of alleles

In diploid organisms a mutant allele of a given gene is called recessive if an individual heterozygous for it is phenotypically like the form homozygous for the original allele. If the heterozygote is like the homozygous mutant form, the mutant is said to be dominant. These terms are convenient but obviously have little real significance, since all degrees of intergradation are found. Thus we refer to phenylketonuria in man as a recessive trait, because heterozygotes seem to be indistinguishable from normals. But, as Penrose (cited from Haldane (130)) has indicated, it appears on detailed study that such heterozygotes are more susceptible to senile dementia than are homozygous normals. In many other cases as well, recessive mutants turn out to be only partially recessive, i.e., intermediate, when a sufficiently refined study is made.

Objective and quantitative measures are needed of the effects of different alleles of a given gene in terms of rates of specific reactions or amounts of specific products formed. While this has been recognized by many geneticists (e.g., 110, 347), very few data are available on which quantitative analyses can be made in such terms. This is in part due to the fact that until relatively recently the number of specific chemical reactions known to be gene controlled has been limited. The most detailed attempt to develop quantitative interpre-

tations of dominance has been made by Wright (344, 346, 347, 348) in connection with melanin formation in the guinea pig. Here there is an obvious limitation, in that the specific reactions by which melanin is formed are not known. In fact, it is not even known precisely what melanin is chemically. In spite of these limitations Wright has been remarkably successful in developing a formal interpretation in terms of theories of gene action which he has formulated.

In the transformation of a substrate to a product genes can obviously play a part in several ways. One of the simplest is that in which the gene is inactive and produces no enzymatic catalyst whatever or an inactive one. Such an allele is known as an amorph. The gene-controlled catalyst (the gene might conceivably itself act directly as a heterocatalyst) may be only partially inactivated (a hypomorph), in which case the dominance relations will depend on the degree of inactivation, the concentration of substrate, and the reaction coefficients. A gene change may alter the catalyst in such a way that an altered product is formed with a modified efficiency in the production of the final character. If the product is entirely ineffective, the mutant allele would be an antimorph in Muller's terminology. If it were of low efficiency relative to the normal product, the mutant allele would be a hypomorph when compared with an amorph but an antimorph when compared to an allele of higher efficiency. It is also theoretically possible (349) that a gene-controlled enzyme may catalyze the transformation of two different substrates. An altered enzyme produced under the guidance of a mutant allele of the gene might have its efficiencies differentially altered with respect to the two substrates. Carried to an extreme, this type of modification might lead from action exclusively on one substrate to similar action on a different substrate, in which case the mutant allele would be classified as a neomorph. For several of these possibilities Wright (346, 347) has formulated the theoretical considerations in mathematical terms; the reader is referred to his papers for details.

Stern and his coworkers (295, 296, 297) have presented an interpretation of the action of alleles of a gene in *Drosophila melanogaster* having to do with the details of wing venation and certain other characters. This involves the assumption of two variable properties of the gene (or the enzyme controlled by it): namely, (*1*) efficiency of transforming substrate to product and (*2*) combining power of gene or enzyme. While this interpretation is at present rather highly speculative, it is just such treatments that state fundamental biological problems in ways in which they can be attacked profitably at a chemical level.

It is probably true that most genes are capable of existing in several forms. Certainly this is so in many particular instances (294). In such cases it is usually found that the several alleles affect the same final character in ways that can be ascribed to quantitative differences in the efficiencies of the alleles or the enzymes they control. For example, in the white series of alleles for eye color in *Drosophila* in which more than a dozen members are known, all alleles are related to pigment formation and can apparently be arranged in a series according to their efficiencies in promoting this process. The pigments produced in the presence of different alleles vary in their absorption spectra,

possibly owing to different degrees of condensation of identical pigment molecules (91). In terms of action, they presumably produce enzymes of different catalytic efficiencies or different amounts of the same enzyme.

In genes determining antigen specificities, several alleles with differing specificities are often known, as has already been pointed out for the A–B blood group gene in man, for the *Rh* gene in man, and for several others. The self-sterility alleles are another example of a similar phenomenon.

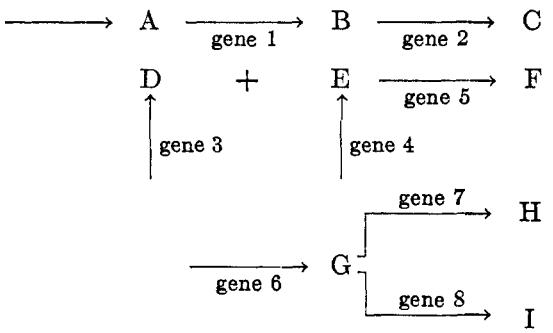
There are several instances in which a series of multiple alleles show independent variability in two or more properties. This is so for the *P* gene in maize, which is concerned with both cob and pericarp colors (red or brown water-insoluble pigments of unknown chemical nature). Here the effects on the color of the cob and that of the pericarp (outer layer of the kernel derived from maternal tissue) vary independently (2) and no simple series can be made covering all effects. A similar relation is found in the alleles of the *A* gene in maize (83, 242, 285), where the effects are: (*1*) on anthocyanin pigments in the aleurone (endosperm cell of the kernel) and other parts of the plant and (*2*) on pericarp pigment. In the *R* series of alleles of maize Stadler and Fogel (287, 288) conclude that there are many alleles and that they can be classified in terms of three independent effects as follows: (*1*) on aleurone anthocyanins, (*2*) on anthocyanins of seedling plant, leaves, husks, glumes, and others, and (*3*) on anthocyanin pigment in the pericarp. They suggest that even more effects may be involved. These three effects vary both quantitatively and qualitatively for different alleles. At least two of the gene effects are subject to independent modification through mutation. An allele promoting both aleurone and plant color may mutate to an allele with either of these effects reduced or lost, but usually both are not lost together (285). A series of alleles of a gene that plays a part in anthocyanin distribution somewhat like that of the *R* series in maize is known in cotton (267).

Genetically the determination of whether two hereditary units are alleles of the same or different genes are made on the basis of (*1*) their interaction in heterozygotes (a/a' *vs.* $a/A\ a'/A'$) and (*2*) whether or not they show crossing over with one another. In the great majority of cases these two criteria agree with each other and give a clear answer. But there are instances in which either or both criteria break down. For example, bar and infrabar are two dominant genes in *Drosophila* affecting the shape of the eye. On the basis of interaction they appear to be alleles, but occasionally they cross over with each other, indicating that they are separate genes (305). Recently similar relations have been found at two other loci in *Drosophila*, one involving recessive alleles of the lozenge gene (225) and the other the dominant character star (176). In two of these cases (bar and star) it has been shown by cytological examination that the original mutant type arose as a result of a duplication of a small section of chromosome inserted in the chromosome next to the original segment with which it is homologous. On this basis the cross-over results find a ready explanation (176). The lozenge example cited may be similar, and no doubt still other situations of the same general nature exist in many organisms.

At the scute locus in *Drosophila* there exist gene forms recessive to their wild-type alleles which interact when put into the same individual to give wild type (32, 75). This suggests that they are non-allelic. However, no crossing over occurs between them, and both are alleles of a third gene form by the two indicated genetic criteria. A case of spurious allelism with at least a superficial resemblance to the scute case has been shown to arise in maize as a result of very small deficiencies produced by a special technique (191). This has been interpreted on the basis that one deficiency involves one gene, a second non-overlapping deficiency an adjacent gene, and a third deficiency both genes. By the genetic test the two short deficiencies are not alleles of each other but are both alleles of the third deficiency. The same situation would arise if recessive genes were substituted for the two small deficiencies. In this model case the deficiencies are so small that, except for particularly favorable circumstances, they could not be seen in cytological examinations.

B. Interactions of non-allelic genes

With gene-controlled reaction systems such as the following:



most of the types of interactions of non-allelic genes observed by geneticists find ready interpretation in terms of changes of normal genes to amorphic, hypomorphic, or antimorphic alleles. For example, genes 1 and 2, or 4 and 5 are complementary. In the diploid offspring of a zygote heterozygous for amorphs for either pair, segregation would give a 9:7 ratio. Genes 2 and 5 or 7 and 8 are independent and the corresponding F_2 phenotypic ratio in a diploid is the familiar 9:3:3:1. Genes 6 and 7 or 6 and 8 show an interdependence such that the expression of 7 or 8 is dependent on the activity of 6. This relation is often spoken of as epistasis, i.e., gene 6 is epistatic to 7. If antimorphic mutant alleles are concerned, the other genetic ratios result. All of these types are known in terms of specific reactions. The A→B→C sequence is represented in the ornithine cycle (page 61). The system in which D and E, each dependent on gene activity for its formation, react to give F is illustrated in the formation of tryptophan through the condensation of serine and indole or the union of pyrimidine and thiazole compounds to give thiamin. The third system, in which the formation of two products depends on a common precursor, is

represented in melanin formation (page 30), in the formation of anthocyanins and anthoxanthins (page 39), and in the production of the two pigment components of the *Drosophila* eye (page 33).

A special case, that of so-called duplicate genes, is found regularly in polyploids and occasionally in diploids. Here the presence of either of two genes permits a given reaction to take place. In polyploids it is clear that such genes with similar functions actually represent physical duplications; the two genes are structurally and functionally identical. Presumably such duplicate genes occasionally arise in haploid or diploid organisms through the occurrence of small duplications. Such duplications are probably of the greatest importance in the process of evolution in providing material from which new genes and new gene-controlled activities can be acquired by the organism as a result of subsequent neomorphic mutation.

Many characteristics of the organism, such as height in man, yield in crop plants, and others, show almost continuous variability as a result of variation in many genes, each of which contributes a small part to the total variability. Such genes are sometimes known under the special term "polygenes" (reviews: 200, 201, 270). There is, however, no need to assume their action to be different in any fundamental way from that of genes such as those which we have been considering (but see Mather (201)).

Heterosis or hybrid vigor is a phenomenon well known to plant and animal breeders. It finds its genetic explanation in the complementary action either of alleles (80, 143, 285) or of non-allelic genes (80, 144). In terms of specific syntheses it can be illustrated by examples in *Neurospora* where two strains, each deficient in the ability to make a specific growth factor, can combine to make a heterocaryon (physiological equivalent of a diploid) in which synthesis of the two essential growth factors is complete (17).

C. Position effect

It is observed in many organisms that genes perform their tasks in a normal manner regardless of their sequential arrangement in the chromosomes. That is, inversions, translocations, and other types of chromosome rearrangements do not usually result in any phenotypic change in the organism carrying them provided there is no net gain or loss in genic material. There are, however, a number of instances known in which the action of specific genes is dependent on their positions with respect to other specific genes (reviews: 73, 224) or to heterochromatin (263, 264). Several such instances are known in *Drosophila* (73, 224, 228) but only one in plants (43). In several cases the effect is reversible, i.e., the original manner of action is restored if the genes are returned to their original position. This reversibility is of course an obvious way of ruling out the possibility that the change in position was accompanied by a true gene mutation. Although several interpretations of the position effect phenomenon have been suggested (92, 224, 295, 305), none is entirely satisfactory in all respects. In most instances the position effect is observed in structural heterozygotes (individuals heterozygous for inversions, translocations, or other

chromosome rearrangements), and Ephrussi and Sutton (92) have recently suggested that physical distortion of the gene due to somatic pairing in such heterozygotes may be responsible for a change in its function. This interpretation was inspired by the recent observation that muscle myosin differs in its enzymatic activity in the extended and contracted states (63, 88). As Ephrussi and Sutton recognize, this interpretation is not completely satisfactory in all respects. Schultz (263) has offered suggestions as to how proximity to heterochromatin may alter a gene's activity, but these are at present highly speculative. Evidently an entirely satisfactory interpretation of position effect in chemical terms lies in the future.

D. Multiple effects of genes (pleiotropism)

The profound consequences to the organism of a single gene substitution have been observed in many cases, and such observations have led naturally to the view that a single gene may do several things, often apparently unrelated. The creeper fowl, studied in detail by Landauer and his associates (163), is a case in point. In birds heterozygous for the creeper mutant allele the long bones are shortened and the mature individual is a disproportionate dwarf. The homozygote dies before hatching from the egg and shows many abnormalities. As these are studied in more and more detail it is found that most of them can be referred to defective yolk-sac blood circulation, which can be detected at 54 hr. of incubation (39). If the eye of a homozygous creeper is transplanted to a normal embryo with normal circulation it develops normally (102). Other effects, however, appear to be determined earlier (251). It seems highly probable that one primary change is responsible for the creeper syndrome.

A similar situation is found in a recessive lethal in the rat studied by Grüneberg and others (86, 95, 130). Here over twenty abnormalities in development have been discerned. Careful study shows that they are all related causally and have their primary origin in anomalous development of cartilage cells. A somewhat similar situation is known in the mouse, where the syndrome of inherited hydrocephaly stems originally from defective cartilage cells (121, 122).

Grüneberg (121) has ably discussed the question of plieotropic gene effects and comes to the conclusion that probably most if not all genes primarily influence one process that is cell- or tissue-specific. This view agrees with the notion that genes produce their effects through controlling specific reactions. These of course would be expected to be cell-specific and tissue-specific rather than organ-specific.

E. Maternal effects

It is observed in a number of instances that the effects of specific genes are evident in cells in which the determining gene alleles themselves are not present. The classical case of this is in the direction of coiling of snail shells, where the inheritance follows the usual mechanism except that the direction of coiling of a given individual is determined by the genetic constitution of its mother

rather than of itself (29, 304, 308). Direction of coiling is apparent at the first cleavage of the egg and evidently depends on the genetic constitution of the egg mother cell before reduction in chromosome number.

From the standpoint of the chemical basis of such genetic carry-over effects, Kühn and Plagge (160) have observed that eggs of the meal moth *Ephestia* which are homozygous for the *a* allele of the *A,a* gene pair carry a^+ hormone which is dependent on the *A* allele for its production. This physical transfer of hormone to the offspring results in pigmentation of the larval skin and eyes. The effect decreases during development and disappears by the following generation. The hormone concerned has been shown chemically to be α-oxytryptophan or a closely related compound (page 34).

Other cases of so-called maternal influence have been summarized by Plagge (233); presumably a similar interpretation involving transfer of gene-dependent substances through the cytoplasm is involved in all of them.

F. Genes and protein synthesis

The available evidence is consistent with the hypothesis that genes are proteins or nucleoproteins and that their two primary actions lie in autocatalytic control of the synthesis of more units like themselves and in determination of the specificities of non-genic proteins. These two functions may be fundamentally one (130, 215, 217, 324). The proteins synthesized under gene guidance may be enzyme proteins, antigen proteins, or possibly structural proteins. Unfortunately there is no general theory of how genes duplicate themselves or control protein specificities that has any support in experimental observation. Since it is not even known with any certainty how a peptide linkage is formed (145), it is clear that all hypotheses having to do with this important question must of necessity be highly speculative. Perhaps the most widely held view (69, 130, 217, 219) is that the gene somehow acts as a master molecule or templet in directing the final configuration of the protein molecule as it is put together from its component parts. Delbrück (69) has advanced a speculative hypothesis as to how such a model-copy system might operate in terms of short-distance interactions between gene and appropriate component parts in such a way as to lead to an accurate copy.

Whether the component parts that are fitted together under gene direction to give the completed molecule are amino acids, amino aldehydes, dipeptides, or units of higher order, it is evident that these too must be synthesized under the direction of many genes, each controlling one specific reaction. This means that while the final configuration is determined by one model gene, many other genes take part *indirectly* in the reproduction of a gene or in the synthesis of a protein molecule. In general, these secondary reactions will be common to the synthesis of many other genes as well.

If the gene acts either directly as an enzyme or indirectly by controlling enzyme specificity, the latter being a necessary assumption for extranuclear enzymes, it is evident that the *primary* effect of a gene mutation will be on a single reaction. Many secondary effects will of course follow, as is known, for ex-

ample, in hereditary failure to oxidize phenylpyruvic acid in man. From an experimental point of view it is not always easy to determine whether or not a particular reaction interrupted when a substitution at a given gene is made is the reaction controlled by the gene in a primary way. As an example, suppose a mutant form were characterized by inability to combine pyrimidine and thiazole to form thiamin. Carboxylase, which contains thiamin pyrophosphate as a prosthetic group, would be inactivated. How would one distinguish between thiamin synthesis and formation of carboxylase protein as the primary action of the gene concerned? If administration of thiamin were to relieve the difficulty, formation of carboxylase protein would be ruled out as a primary effect. Thiamin synthesis presumably involves an enzyme catalyzing the union of the pyrimidine and thiazole moieties. It is possible that the "S" factor of Kidder (148) serves as a prosthetic group in this enzyme. If so, and if administration of "S" factor were to enable the mutant form to synthesize thiamin, the primary gene action would lie in some reaction in the synthesis of "S" factor; if not, the primary action would be in the production of the protein component of the enzyme for thiamin synthesis. Such an analysis depends on the prosthetic groups or coenzymes being obtainable for the enzymes of the reactions that might *a priori* be primary. Unfortunately, of course, this is not often the situation.

VIII. VIRUSES AND PLASMAGENES

Almost as soon as the discovery of viruses became known to the world, Troland (324) appreciated that fundamentally they were very "gene-like." His vision is indicated in the following quotation taken from his paper published in 1917, two years after Twort first demonstrated filterable agents and the same year in which d'Herelle published independent confirmation:

> There is considerable evidence that free autocatalytic enzymes exist in our biological universe even at the present day. Such an hypothesis would serve to account for the specific contagious diseases, such as measles, rabies, and smallpox, which have been demonstrated to possess "filterable viruses."

This quotation assumes added significance when it is pointed out that it was Troland's belief that genes and enzymes are intimately related. Since Troland's paper, the evidence for basic similarity between genes and viruses has steadily increased (217, 219). We may summarize these similarities as we know them today as follows:

(*1*) Both lie within the same size range. Viruses cover a considerable range in size (11, 290, 291). While estimates of the sizes of genes are relatively crude (218), they suggest that genes are of the same order of size as tobacco mosaic virus, a medium-sized virus.

(*2*) Both appear to be or at least to contain nucleoproteins. The evidence that this is so for genes has been summarized—it consists of chemical analysis of chromatin and the observation that the ultraviolet wave-length mutation spectrum parallels the absorption spectrum of nucleic acids. Purified viruses have been subjected to direct analysis and shown to be ribose nucleoproteins

with the protein components more complex than protamines or histones (11, 290, 291). Bacterial viruses likewise appear to be ribose nucleoprotein in nature (review, 70). Genes appear to contain desoxyribonucleic acid rather than the ribose analogue, but this difference is not certainly established.

(3) Both have the property of self-duplication in the proper environment, i.e., in actively metabolizing living cells of the proper genetic constitutions. A difference in the two entities is seen in the fact that genes usually duplicate themselves only once per cell generation, while viruses are not subject to this regularity in their multiplication.

(4) Genes and viruses are both subject to mutation, i.e., changes in their properties other than those permitting self-duplication. Mutation in genes has already been discussed. The results of the process in viruses (including phages or bacterial viruses) are often observed (reviews: 11, 70, 162, 196, 290). In both viruses and genes mutation is known to be a reversible process (11, 70). In viruses it is known to involve a change in immunological specificity and a change in amino acid composition in some instances (11, 155, 290, 291). Inactivation in both genes and viruses by x-rays is known to be a "single hit" phenomenon, i.e., to be brought about by single ionizations (70, 118).

(5) Both genes and viruses influence metabolic processes in ways that often appear to be similar. It is interesting in this connection that no enzyme activity is known in cell-free viruses (70).

The origin of viruses is not known with certainty in any specific case, but on logical grounds it seems most reasonable to assume, as Darlington (67) and others have, that they arise from normal cell proteins by some change analogous to mutation. Indirect evidence for this comes from the fact that there exist virus carriers in both higher plants and bacteria (so-called lysogenic strains) which show no detectable symptoms of virus presence. Salamon and Le Pelley (256) have demonstrated that all strains of King Edward potato carry para-crinkle virus, which is without detectable effect. When the virus is transferred to certain "susceptible" varieties by grafting, symptoms of disease develop. Since no way other than grafting is known of transmitting para-crinkle virus, it is probable that the agent arose in the King Edward variety itself (67). Somewhat similar situations are known in bacterial viruses. White (334) has found that most if not all Indian strains of *V. cholerae* carry a certain virus but show no apparent effects of it. All tested Chinese and Japanese strains of the bacterium, on the other hand, are lysed by this virus.

Plasmagenes are postulated self-duplicating cytoplasmic units which, like genes and unlike viruses, are normal cell constituents (79, 269). Because it has not heretofore been possible to demonstrate the existence of these units in a manner as unequivocal as those used in the demonstration of genes and viruses, most geneticists and other biologists have been skeptical as to whether such units, comparable in autonomy to genes, really exist. The evidence is now sufficient, however, to justify serious attention to the possibility of gene-like units in the cytoplasm. Actually, of course, the distinction between viruses and plasmagenes on the basis of one being normal and the other an abnormal cell

constituent may be quite artificial. Is para-crinkle virus a normal or an abnormal component of the cell of King Edward potato? Incidentally, instances of this kind suggest that viruses may be acquired by one species from another in which they are *normal* cell components. It seems most probable that there are "viruses" carried by species for which no tester strains exist. These would most certainly be classed as normal cell constituents if there were any way of identifying them which was not dependent on the production of disease.

A second distinction between viruses and plasmagenes is that one is infectious and the other not (67). This too is obviously an artificial basis of separation. Natural transmission might well depend solely on whether or not a suitable insect vector were present.

Plastids in plants are known to have a certain autonomy in their transmission as cytoplasmic units (241). Often defective plastids arise by a mutation-like process which, regardless of the genetic constitution of the nucleus, is perpetuated indefinitely through self-duplication (138). This is not to imply in any way that plastid development and functioning are independent of gene control, for genetics has shown in hundreds of cases that such a control does exist. Regarding the relation between the two factors, gene control and self-duplication of plastids, Rhoades (243) has recently described a most instructive case. In maize a recessive gene allele is known to induce frequent "plastid mutations" to a defective type. Once these have arisen under the influence of the gene concerned, they perpetuate their kind and the change that produced them cannot be reversed by replacing the mutant form of the gene with its normal allele.

That plastids and their supposed precursors, mitochondria, contain self-duplicating units in some respects like genes is indicated by two other types of evidence. Claude (52) and others (76) have shown that these cytoplasmic structures contain ribose nucleoproteins. Woods and Du Buy (342) have obtained evidence that in certain types of plant varigation due to self-duplicating defective plastids, the defective agent can be transmitted from one part of a plant to another through grafting. Only one positive case of transmission was obtained, however, and because of this and the possibility of alternative interpretations, confirmation is needed before this finding can be accepted without question.

A number of instances of apparent cytoplasmic inheritance are on record. One of the most thoroughly studied of these involves the flowering plant *Epilobioum* (208, 209, 210). Here reciprocal crosses between species and between different races of a single species show differences in the hybrid plants of the first generation. Behavior in back-crosses to the parents is consistent with the assumption that cytoplasmic entities transmitted through the egg are involved. It seems probable that these are in some respects like para-crinkle virus in that their effects depend on the nuclear constitution of the plant in which they are found. They are *normal* in plants of one genetic constitution but not in those of another. They differ from viruses in not being infectious under the conditions in which they have been studied.

Sonneborn (279, 280) has reported experiments on "killer" and "sensitive"

strains of the protozoan *Paramecium aurelia*, which he interprets in terms of a substance of the cytoplasm which multiplies under gene control. Animals carrying the dominant allele of the *K* gene can produce this substance (known as kappa) provided some is already present in the cytoplasm, but they cannot initiate its formation. It can be physically transferred to an animal genetically capable of making it but lacking the primer, and it will thereafter be multiplied in step with the vegetative reproduction of the animal. The hypothetical substance kappa is apparently "adsorbed" by the *K* allele of the killer gene when this is present in the macronucleus of the animal.

A situation similar in at least certain respects is found in the transformation of the types of pneumococci under the guidance of a specific nucleic acid (6, 279). Here apparently the nucleic acid supplied the bacterium initiates a process by which more nucleic acid of the same type specificity is synthesized. If the nucleic acid is a gene (349), or a plasmagene, it must be capable of direct self-duplication. On the other hand, if it is an inducer of a specific mutation, the production of more nucleic acid of the same type would be a less direct process.

Spiegelman, Lindegren, and Lindegren (281a) have reported that an enzyme concerned with the splitting of the disaccharide melibiose in yeast is formed under the direction of a specific gene (or two under their interpretation) but that, once formed and in the continued presence of the substrate melibiose, the enzyme continues to be formed in the absence of the active allele of the gene initiating its production. Apparently the enzyme is self-duplicating in the presence of its substrate. The enzyme can be thought of as a kind of plasmagene, but one of a special type, since it reproduces only in the presence of the substrate.

A partly hypothetical chemical model of self-duplication of cytoplasmic elements can be constructed in terms of what we know about the formation of certain enzymes. As is well known, pepsin is formed autocatalytically from an enzymatically inactive precursor pepsinogen (review, 223). Both enzyme and precursor are known in crystalline form and both are proteins. While presumably closely related chemically, they differ from each other both serologically and in crystal structure. If we assume a situation in which pepsin were synthesized under the direction of a specific gene, without going through pepsinogen, we would have a standard gene–enzyme relation. It is conceivable that the pepsin-specific gene could mutate to a form which, instead of directing the synthesis of pepsin, determined the production of pepsinogen. So long as pepsin remained present, there would be no selective disadvantage in such a mutation. However, if for some reason the supply of pepsin were temporarily exhausted, the organism would remain pepsin deficient. If then a small amount of pepsin were introduced into the cell, it would multiply even in the absence of the gene which originally directed its synthesis and we would have a situation analogous to that reported by Spiegelman *et al.*, except that no substrate would be required for enzyme self-duplication.

It is becoming more and more evident as our knowledge increases that genes,

enzymes, antigens, plasmagenes, viruses, and other proteins have many properties in common. It would not be surprising, then, if with slight change in structure or in cellular environment one of these should change to another. Examples are continually being found in which traditional distinctions break down. For example, the muscle protein myosin is now thought to be both a structural protein and an enzyme (63, 88).

IX. EVOLUTIONARY CONSIDERATIONS

Troland (323, 324), Alexander and Bridges (1), Oparin (226), Plunket (235), Koltzoff (156) and others have speculated on the question of how the first autocatalytic particle, the "protase" of Troland or the "protogene", arose from materials not possessing this essential property of living things. The conclusion seems almost inescapable that this most significant step in the origin of life took place in an environment in which many organic molecules had spontaneously arisen from inorganic precursors and remained intact because of absence of organisms to break them down (226). Protogenes presumably had only the property of autocatalysis, not that of heterocatalysis. If we assume that the property of heterocatalysis was acquired by protogenes in subsequent evolutionary steps, we can imagine the gradual evolution of symbiotic systems of increasing complexity in which protogenes became genes with specialized heterocatalytic functions (1). This view underlies a possible interpretation of the evolutionary development of complex syntheses in which the intermediate products serve no use. It is commonly assumed that such systems of synthesis evolve from simpler to more complex. On this basis it might be assumed that in arginine synthesis the first step is the reduction of nitrate to nitrite. Nitrite is further reduced to NH_4^+ nitrogen. There follows a series of reactions of unknown nature, leading to ornithine. This is converted to citrulline, which in turn is transformed to arginine. But for this series of reactions to evolve from the nitrate end of the series it is necessary to suppose either (*1*) that all the steps arose simultaneously, (*2*) that the ability to synthesize each compound in the series conferred a selective advantage on the organism, or (*3*) that the individual steps arose independently and persisted without advantage until combined by chance into a single organism. Each of these possibilities is individually highly improbable, as is any combination of them. The last implies sexual reproduction, which presumably arose late in the process of evolution of organisms. Horowitz (135a) has recently suggested that the evolution of series of reactions leading to a final product useful to the organism, or system of symbiosing genes, may have evolved backward from the final compound in a manner in which each successive step would have a selective advantage to the system. Thus, if the original protogene took arginine from the medium in which it reproduced, a mutation leading to catalysis of the reaction citrulline to arginine would have a strong selective advantage if both citrulline and arginine were present in the medium. In a similar way mutations leading to catalysis of the reactions by which ornithine is converted to citrulline would take the system of genes a step closer to independence of preformed organic molecules

and would confer an additional advantage. Each new mutation in the system would have a chance of adding a new reaction to the series.

It should be pointed out that there is nothing in the hypothesis of backward evolution of reaction systems incompatible with the occurrence of evolutionary steps in the opposite direction. The original final product in the ornithine series might, for example, have been citrulline with arginine substituted in a later evolutionary step. Whatever was the nature of the first autocatalytic system, its component parts must have been taken from the environment. If these were organic molecules of any type, the backward evolution in the series to simpler precursors would appear to be a probable process.

Since mutations in genes leading to their loss or inactivation are more frequent than the reverse process, reactions of no advantage to the organism are not expected to persist indefinitely. This means that saprophytic plants, their counterparts among animals, and all parasitic forms which find themselves continuously in the presence of certain biologically significant compounds would be expected to lose their ability to synthesize these compounds. That such evolutionary specialization has indeed taken place in many bacteria, protozoa, and other organisms is clear from the work of Lwoff (190), Knight (154), Schopfer (262), and others. Unless intermediates in the end products no longer synthesized are useful to the organism in independent ways, all steps in a given synthesis will tend to be lost eventually. In this way specialization through loss of function will be essentially irreversible. The possibility must be kept in mind, however, of the evolutionary reconstruction of a reaction system in the manner in which it originally arose.

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