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PHYSIOLOGICAL ASPECTS OF GENETICS[1,2]

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In its concern with the physical, chemical, and biological nature of the specifications which living systems transmit from one generation to the next, genetics has come to play a central role in modern biology. Progress is being made so rapidly that it is almost impossible for a single individual to keep abreast of advances on all fronts. This review is prepared for physiologists and others who are not primarily specialists in genetics. It will attempt to survey in a general way some of the advances that seem to the author to be of particular interest and importance. In no sense will it cover all of significance in genetics that has happened in the three years since the preparation of the previous review of genetics for this series (1). Fortunately a number of excellent summaries of progress in particular branches of genetics have recently appeared or are in preparation. These, rather than more technical papers, will often be cited as convenient sources of additional information. Pontecorvo's Jesup Lectures (2) provide an excellent survey of newer approaches to genetic analysis. Ravin (3) and Wheeler (4) have recently reviewed work on the genetics of bacteria and fungi, and Levinthal has surveyed the general situation, especially for physicists and other non-geneticists (5). Fincham (6) has in press a summary of the genetics of enzyme activity.

It is widely believed that the primary genetic material in tobacco mosaic and other viruses of similar composition is ribonucleic acid (RNA) and that in other viruses and all cellular organisms it consists of deoxyribonucleic acid (DNA). The most direct evidence that these are correct interpretations is found in the observations that the protein of tobacco mosaic virus can be completely removed and discarded without destroying infectivity (7, 8), that bacteria can be genetically transformed with pure DNA from related but genetically different bacteria (9), and that in isotopically labeled bacteriophages only DNA is carried over from one generation to the next (10).

The evidence is strong that, with some exceptions, genetic DNA occurs in the form of Watson-Crick double polynucleotide helices, in which the chains are oppositely oriented and in which nucleotides are specifically paired

[1] No attempt is made to cover the entire literature of the subject nor is this review confined to the literature of any fixed period. The practice of referring to review articles or to only the most recent of several papers on a given subject necessarily means proper credit for facts and ideas will often not be given. More documentation will be found in papers cited and these should be referred to by readers interested in more than a general survey of the areas covered.

[2] The author is grateful to Mr. Robert W. Hedges, who helped with the literature survey on which this review is based, and to colleagues and friends who have generously allowed him to see manuscripts of papers in press.

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through hydrogen bonding between complementary bases, adenine with thymine and cytosine with guanine (11). Sequences of nucleotides are believed to be responsible for the genetic specifications of RNA and DNA.

These assumptions provide a background for examining possible answers to four questions: (*a*) By what mechanisms are nucleic acids replicated? (*b*) How are the specifications of nucleic acids used in development and function? (*c*) What is the molecular basis of mutation? (*d*) What is the coding system by which nucleic acids carry genetic information?

Because an understanding of recently discovered features of the life cycles of bacteria and bacteriophages is essential to a proper appreciation of the ways in which these organisms have been used in attempts to answer questions like the above, brief accounts of bacterial conjugation, lysogeny, and transduction are given below.

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Bacterial Conjugation

In addition to their well-known advantages of easy culture, small size, and rapid reproduction, some bacteria possess novel mechanisms for exchanging and recombining genetic material. One of these is *Escherichia coli*, in which conjugation was discovered in 1946 by Lederberg & Tatum (12). It is a remarkable process, the understanding of which has involved the efforts of many investigators (13, 14).

There are donor and recipient strains of *E. coli*. Individual cells usually contain two, three, or perhaps more nuclei, each of which includes a single chromosome, and ordinarily all nuclei are genetically identical. Under suitable conditions donor and recipient cells conjugate pairwise. A delicate conjugation tube forms between the partners. Through this the chromosome of one nucleus of the donor cell migrates always "head" first, in a manner much like a rope being pushed or pulled from one box to another through a short connecting pipe. The migration is slow, requiring some 2 hrs. for completion. During this interval, the partners often break apart, with the result that only a part of the donor chromosome enters the recipient.

Whatever the length of the donated chromosome segment, it is capable of genetic recombination with a chromosome of the recipient, perhaps by a process like crossing over in higher organisms. If the transferred donor chromosome or a segment of it differs genetically from its counterparts in the recipient, genetic modification will result from incorporation.

In subsequent cell divisions of the recipient, the modified nucleus will be segregated from its unmodified sisters. Odd fragments of chromosomes left over in the process will be eliminated, probably in inviable or slowing growing segregant cells (15).

This amazing process makes it possible to determine gene order in bacterial chromosomes in at least three ways. With several gene differences between recipient and donor, linear arrangement of genes can be inferred in the usual way from relative recombination frequencies. Artificial interruption of the mating process, e.g. in a Waring blender, at known times, reveals the

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order and spacing of genes from head to tail with remarkable precision. The third method, breakage of the donor chromosome by disintegration of incorporated P^{32}, will be discussed later. Results of the three methods agree.

Sequence of entrance marker genes and position of the head vary among different donor strains as though all were derived from a single ancestral form in which the genes have fixed positions in a ring chromosome. The ring appears to be opened in various positions to give rods, with one of the broken ends becoming the head. It has been suggested that a special kind of "episome" attaches to the ring chromosome resulting in an adjacent break with the end on which the episome remains, becoming the tail (16).

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Lysogeny and Transduction

Bacteria often carry latent phages that are only occasionally released as particles capable of reinfecting sensitive cells. Phages of this type were not, however, fully appreciated, either as regards their general biological significance or their usefulness as experimental material, until about a decade ago when Lwoff took an active interest in them and with his colleagues succeeded in showing that some strains of lysogenic bacteria can be induced by radiation or otherwise to convert their prophages into vegetative phages which then multiply and complete a lytic cycle (17). Unlike virulent phages, temperate phages released from synchrony with their hosts are able to lysogenize sensitive bacteria as well as lyse them, these alternatives being to a considerable extent dependent on conditions of culture of host bacteria.

More than a dozen temperate phages have been studied in *E. coli*, some inducible, others not (18, 19). In its prophage state the total DNA of a temperate phage becomes attached to a bacterial chromosome, one per chromosome, at a particular locus. The attachment is probably not by replacement of bacterial DNA or by insertion. The various temperate phages are differentiated by their specific positions of attachment to the bacterial chromosome as prophages, these being determinable by the methods described above for locating bacterial genes and by the fact that each in its prophage state inhibits vegetative multiplication of homologous phages but not of others. The DNA of a temperate phage can be shown to be made up of segments which determine such properties as ability to lysogenize (i.e. virulence vs. non-virulence), degree of virulence, position of attachment as prophages, and ability to carry out the several synthetic processes involved in conversion to infectious particles following induction.

In addition to immunizing its host against vegetative multiplication of homologous phages, presence of a prophage may alter the properties of the host in other ways—antigenically, in toxin production, and in enzyme synthesis. There are several excellent recent reviews of lysogeny, of the genetics of temperate phages, and of phage genetics in general in which additional information and documentation can be found (20 to 24).

Another important property of some temperate phages, discovered by Zinder & Lederberg (25), is their ability to transport small segments of bac-

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terial DNA from the cells in which they mature to the cells they subsequently lysogenize. The bacterial DNA so carried is capable of replacing homologous DNA of the recipient cell and thus bringing about genetic change. Although this may occur in only one in a million cells for a given gene, the frequency can be measured under conditions in which only changed cells multiply.

Since the DNA segments so transferred are small relative to the total bacterial DNA and since a single phage apparently seldom carries more than one segment, it follows that if two genetic markers are found to be transferred together with any appreciable frequency ("co-transduction"), they must be closely linked. If three markers closely linked in the order *abc* are being followed, replacement of a single segment of DNA of the recipient can lead to incorporation of any one of the following combinations of markers: *a, c, ab, bc,* or *abc*. But incorporation of *ac* without *b* cannot be accomplished by replacement of a single continuous segment and will therefore occur with a very much lower frequency. Hence, by measuring the relative frequencies of the above six possibilities on selective media, the linear order and relative spacing of the three markers can be determined. Especially in the hands of Demerec and his co-workers (26), this method of genetic analysis has proved to be a powerful one.

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Replication of DNA

The Watson-Crick structure immediately suggested to its authors that replication might be accomplished by separation of paired nucleotide chains, with each chain remaining intact and serving as a kind of template against which new complementary partners were built. In contrast to this "semiconservative" scheme, two other possibilities have been considered, a "dispersive" one in which segments from old and new chains are assumed to become intermixed and a fully "conservative" one in which double helices are postulated to serve directly as templates for synthesis of ribonucleoprotein molecules which in turn direct synthesis of wholly new double DNA chains (21).

Several ways of following isotopically labeled DNA during replication have been employed to determine the manner in which replication actually occurs. The star method of Levinthal (27) consists in following P^{32}-labeled DNA from parent to offspring phage particles by imbedding them in a "nuclear" emulsion and determining the distribution of label by observing ion tracks originating from single phages. This suggests that about 40 per cent of the DNA of a phage gives the distribution expected on the semiconservative model, the remainder being dispersed. The P^{32} "suicide" method of following DNA in phage multiplication indicates that a somewhat smaller fraction is conserved but does not clearly distinguish between the fully conservative and semiconservative schemes (28).

A third method involves labeling DNA with N^{15}. By the density-gradient method, DNA fully labeled with N^{15} can be cleanly separated from N^{14} DNA. At the same time its amount, density, molecular weight, and homogeneity

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can be measured. Starting with bacteria in which substantially all DNA was fully labeled with N^{15}, Meselson & Stahl (29, 30) observed that after doubling of the population in an N^{14} medium—i.e., one generation—all DNA molecules (mol.wt. ca. 7×10^6) were intermediate in density between N^{15} and N^{14} controls. As seen in Figure 1, this is the result expected on the original semiconservative model of Watson & Crick. After two generations in light medium, half the molecules were found to be light and half intermediate. A third-generation population contained the predicted three-fourths light and one-fourth intermediate molecules.

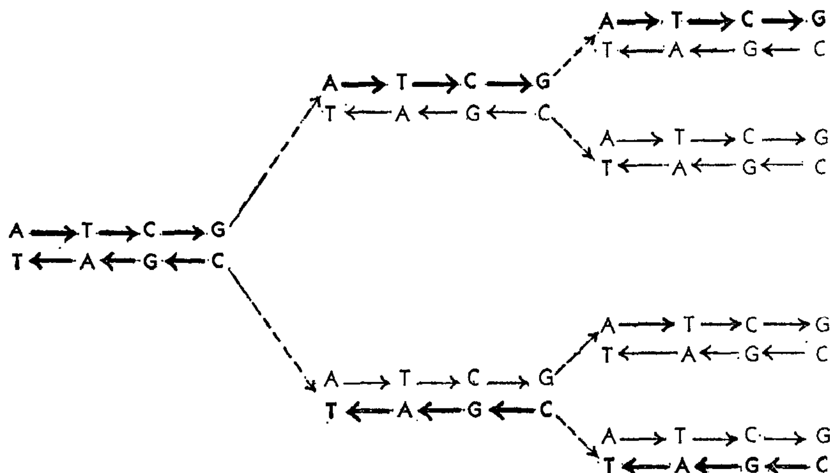

FIG. 1. Representation of DNA replication. *A*, *T*, *C*, and *G* represent adenine, thymine, cytosine, and guanine nucleotides respectively. Arrows indicate polarity as determined by 3–5 sugar linkages. Original parental chains shown in bold face type; new chains in light face. If only original chains labeled, molecules of first generation will be hybrid, those of second generation half hybrid and half unlabeled.

First-generation DNA of intermediate density treated with strong sodium chloride for several minutes at 100°C. separated into heavy and light units of approximately half the molecular weight of the untreated material. These were presumed but not rigorously proved to be single chains.

The results of following N^{15} labels are beautifully consistent with the original Watson-Crick hypothesis of DNA replication. Although it is not easy to see how they could be accounted for by a different mechanism, this possibility must be kept in mind.

A spectacularly successful approach to the problem of DNA replication has been made by the Kornberg team (31 to 33). This group has succeeded in devising a system in which DNA multiplication occurs *in vitro* in a relatively simple solution containing adenine, thymine, cytosine, and guanine nucleoside triphosphates; a polymerizing enzyme from *E. coli;* magnesium

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ions; a suitable buffer; and a DNA primer. All four nucleosides must be present. The ratio of adenine plus thymine to cytosine plus guanine nucleotides of the product is like that of the primer and thus is not dependent on the relative concentrations of nucleosides reactants (32).

Unfortunately the synthesis does not go on indefinitely. After an increase of tenfold or so, net synthesis ceases. This is believed to result from DNase as an impurity in the polymerizing enzyme. Breakdown of DNA in this way would account for the fact that so far no biologically active product has been demonstrated when DNA with transforming activity is used as a primer. With DNA primer, synthesis begins at once and reaches a maximum amount of product in two or three hours. Without primer, essentially no synthesis occurs during the first three hours but, as reported by Lehman *et al.* (32), there then occurs synthesis of a spontaneously formed double-chain copolymer made up exclusively of adenine and thymine nucleotides, each chain containing both adenine and thymine nucleotides. If this spontaneously-formed copolymer is used as a primer in a new system containing all four nucleotides, synthesis of more adenine-thymine copolymer begins without lag. The product, like the primer, contains only adenine and thymine nucleotides.

The combined Meselson-Stahl and Lehman *et al.* results constitute a strong case for direct replication of DNA according to the original Watson-Crick hypothesis and without obligatory intervention of RNA or protein corresponding in specificity to the DNA being replicated. It is of course possible that the *in vitro* and *in vivo* mechanisms differ in significant respects, but it would be strange indeed if there were two fundamentally different ways of accomplishing this remarkable end result.

The spontaneous appearance of the adenine-thymine copolymer in the relatively simple Kornberg system—a copolymer that presumably can undergo mutation to form a true DNA—lends considerable credence to the hypothesis that such molecules played a decisive role in the earliest stages of organic evolution (34).

It has recently been reported that the DNA of the two small phages *S*13 and $\phi X174$ is probably in the form of single polynucleotide chains consisting of only about 5500 nucleotides (35, 36). This conclusion is based on studies of physical properties, P^{32} inactivation, and nucleotide ratios. How is such DNA replicated? The answer is not known. If it is by the Watson-Crick mechanism, there must be some remarkable way of discarding one chain of a complementary pair—and always the same one. If not, one wonders if it and RNA might be multiplied in similar ways.

Replication of RNA

There can be no doubt that RNA of tobacco mosaic virus is capable of both carrying genetic specification and being replicated. The RNA core of a virus rod is by itself capable of transmitting to a host cell all the information needed to produce more viral RNA as well as coats of the same protein spec-

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ificity as that of the intact virus particles from which the RNA was prepared (7, 8).

Is tobacco mosaic virus RNA replicated by some direct method like that postulated for DNA, or in an indirect manner with DNA or protein, or both, serving as intermediaries or collaborators (21, 37)? Direct replication of the cytoplasmic RNA's of cellular forms apparently does not occur, for if it did, the result should be a readily detectable system of cytoplasmic genes. Furthermore, it has been shown that whereas P^{32} decay in bacterial DNA produces lethal mutations, the same result is not observed as a result of the decay of this isotope incorporated in RNA (28). It therefore seems likely either that RNA in general does not replicate like DNA or that the RNA of tobacco mosaic virus differs in its replicative potentialities from that of cellular forms.

The structure of RNA is not known in the same detail as that of DNA (38). It appears not to form stable double helices *in vivo*. However, like DNA, it is synthesized *in vitro* in a system containing appropriate ribonucleoside di- or triphosphates and a polymerizing enzyme, and otherwise much like that in which DNA is replicated (39, 40). This synthesis appears to differ importantly from that of DNA. Polymers of a single nucleotide are synthesized if only a single nucleoside precursor is provided. No primer is necessary and although the source of the enzyme and the primer, if the latter is added, appear somehow to influence the composition of the product, there is considerable doubt as to whether this system is capable of synthesizing biologically specific RNA.

Replication of Chromosomes

The chromosomes of the protista, metazoa, and multicellular plants are orders of magnitudes larger than Watson-Crick helices, the former being readily visible in the light microscope while the latter are only about 20 A in diameter. There is likewise very much more DNA per unit length of a single chromosome strand than could be accounted for by a single longitudinally oriented DNA double helix. The genetic observation that a first or second cell-generation descendant of a chromosome in which a gene mutation has been induced often carries only mutant genes makes it highly unlikely that the chromosome contains a bundle of many genetically homologous DNA helices.

Possible chromosome structures have been reduced in number by determining the distribution of isotopically labeled DNA during chromosome replication. Taylor and collaborators (41 to 43) have used the following technique: plant root tips are allowed to take up tritium-labeled thymidine over a short period so that newly synthesized DNA of replicating chromosomes is labeled. Daughter chromosomes produced in such cells are labeled throughout their lengths as shown by autoradiography. In the next chromosome generation during which no label is present, the two sister chromosomes, derived from a labeled parent, differ in their labeling. If one daughter is labeled throughout its length, the sister will be unlabeled. On the other

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hand, if one daughter is labeled over part of its length the other will be unlabeled in the homologous segment. The second situation, reciprocal labeling of segments of sister chromosomes in the second division cycle, is presumed to result from crossing over between sister chromosomes. This would not be detected in the first division cycle, for both units of both chromosomes are involved in the exchange. The pattern of such sister chromosome exchanges indicates that the two units of a chromosome are not identical. The result is as though they differed in the same sense in which the two chains of a DNA helix differ in the orientations of their sugar-phosphate linkages.

In the hands of some investigators, labeling experiments of this type have not given such apparently clear-cut results (44). Since the systematic and regular distribution described above has been observed in three distantly related species of plants, it is difficult to believe that experimental errors

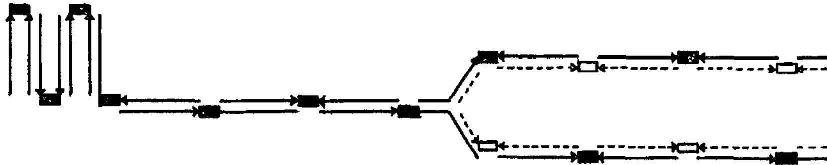

FIG. 2. Chromosome structure according to Freese. At left, folded DNA double chains (antiparallel arrows) and protein interconnections (rectangles); center, in linear arrangement; at right, parental chains separated and new daughter complements being formed. Polarity of DNA chains indicated by arrows.

should have conspired in all cases to give the regularity (45). The reviewer therefore considers it highly probable that the facts are substantially as indicated in Figure 1.

What is the structure of a chromosome element that permits it to carry so much DNA and yet behave like a single Watson-Crick DNA chain, both genetically and in the clean separation of old and new DNA in its next cycle of replication? Freese (46) has proposed a model that appears to meet the specifications. This assumes alternating segments of DNA helices and non-DNA connecting units that may well be protein. The DNA is presumably folded accordion-fashion in the chromosome (Fig. 2). Each DNA segment presumably corresponds to a bacterial DNA molecule as investigated by Meselson & Stahl (29).

Zubay & Doty (47) have examined deoxyribonucleic acid from calf-thymus nuclei extracted in what is believed to be its native state. The molecular weight is about 18.5×10^6, approximately twice that of the DNA alone or of bacterial DNA as determined by Meselson & Stahl. The structure appears to be that of a Watson-Crick double helix with histone occupying the wide groove. It would seem probable, therefore, that if the Freese model is substantially correct, the DNA segments are DNA plus protein.

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Genes and Proteins

The concept that genes determine the properties of proteins had its beginnings soon after the rediscovery of Mendel's paper in the work of Garrod (48) and others. It is of interest to note that it has taken geneticists, biochemists, and physiologists almost half a century to appreciate fully Garrod's interpretations, and it was not until 1958 that the six enzymes involved in the pathway by which phenylalanine and tyrosine are metabolized via homogentisic acid were identified and homogentisic acid oxidase activity shown to be absent in an alkaptonuric activity (49).

The concept that enzyme specificity is somehow genetically determined is now so widely accepted that additional examples are of interest not so much per se but mainly if they add to our understanding of the relation between gene and enzyme, if they can be used advantageously as genetic markers, or if they serve the biochemist as alkaptonuria served Garrod in working out the chemistry of metabolism. In the latter connection, Vogel & Bonner (50) have prepared a most useful summary of the methods and accomplishments of using mutant types of microorganisms in elucidating the pathways by which vitamins and amino acids are synthesized and metabolized.

Genetic determination of enzyme specificity is of course one aspect of the more general problem of protein synthesis. What is the role of genetic material in this process? In the case of nucleoproteins of viruses and chromosomes it is not unreasonable to assume that genetic nucleic acids serve directly as templates to specify sequences of amino acids (47, 51). Although in the cellular forms in which protein synthesis occurs extensively in the cytoplasm the transfer of specificity must be less direct, there are now known so many instances in which properties of proteins of various kinds are subject to genetic modification that one can generalize with confidence that such transfer does occur for all biologically significant proteins.

Even had it not been done adequately elsewhere (52 to 54), it would not be appropriate or feasible to review here the detailed evidence on which are based current views of cytoplasmic protein synthesis. In brief summary, it is assumed that specific segments of nuclear DNA—genes by the definition followed in this review—transfer specificity to RNA, possibly by serving as templates in directing its synthesis (38, 55). RNA molecules made in this way are believed to migrate from the nucleus to the microsomes of the cytoplasm (56).

In microsomes, each of which may carry sufficient RNA to specify only a single protein, the RNA is postulated to serve as a final template in ordering the amino acids previously activated and attached to segments of "soluble" RNA. One of the many important unanswered questions about the cycle of synthesis in microsomes is whether there is a specific soluble RNA for each amino acid, obviously a question of basic importance from a genetic point of view (57 to 59).

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The concept that, aside from self-direction in replication, genes have single primary functions and that these often consist in the control of specificity of proteins has developed gradually over a period of several decades (6, 60). The observations on which it was originally based were mainly of two kinds. (*a*) Mutant genes of independent origins associated with a single easily definable function—such as a biochemical reaction, activity of an enzyme, or other specific properties of a protein—were found to be allelic in many instances. (*b*) The deleterious results of many gene mutations were found to be essentially corrected by supplying the organism with a single metabolite. It is obvious that decisive evidence for or against this concept must come from detailed descriptions of the genes themselves and of the proteins or other macromolecules whose specificity they determine. It is an encouraging fact that just such evidence is now being obtained by a number of groups of investigators (5). The conclusions toward which these efforts point can perhaps best be indicated by briefly reviewing specific areas of investigation.

Genetic fine structure in bacteriophages.—The concept of genetic material as a series of linear templates of nucleic acid—the nucleotide sequences of which provide specificities transferable to other linear molecules, like proteins—predicts that there should be many mutational sites within each such template. Although there were indications that this might be so for certain loci in maize, *Drosophila*, *Aspergillus*, *Neurospora*, and other organisms (2), it was Benzer's investigation of the rII locus of T4 phage that most clearly demonstrated by genetic means the existence of the predicted internal structure of a single genetic unit (61, 62).

Benzer investigated a group of some 280 mutants alike in host range and plaque-type properties. Except for a few unsuitable for investigation, all were found to be located within a region of the phage-linkage map a few recombination units long. With especially devised techniques of measuring very low frequencies of recombination, it was shown that recombinations were in fact obtained for many pairs of mutants within the group and that the relative frequencies of these permit the construction of a linear map within the rII locus. Some mutant types behaved as though they were deletions of substantial segments of the locus.

By infecting suitable single bacterial cells simultaneously with two rII mutants of independent occurrence, it could be determined whether their defects were complementary in the sense that the two together could complete development in a host in which separately neither could do so. By this test all mutants investigated could be assigned to one of two subgroups. By mapping techniques, all of those within one subgroup were located in a continuous segment while all those in the second subgroup were found to lie in an adjacent but separate subgroup.

The genetic unit within which no two mutants show functional complementation in a diploid, a heterocaryon (two or more nuclei of different genetic types within a common cytoplasm—two in a simple test for complementa-

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tion), or in double infections in the case of viruses would be defined as a gene by some geneticists. Benzer proposed that the more precisely defined term "cistron" be used for such a unit. It will be seen later that the operational definition of a cistron is not always as simple as it is for rII mutants of T4 phage.

Leaving aside terminology, it is clear that the genetic properties of rII mutants are beautifully consistent with the DNA concept. It is unfortunate that the rII system has not so far proved favorable for a chemical study of the products of gene action.

It was soon shown that multiple mutant sites within single functional units of genetic material were not limited to viruses. The yeast *Schizosaccharomyces pombe*, for example, shows a similar multiple structure for a gene concerned with adenine synthesis (63). Other examples are discussed below.

Genetics of Salmonella.—Analysis of the genetic apparatus of the bacteria *Salmonella typhimurium* by the transduction technique shows that in this organism, too, individual units of genetic material with discrete functions possess many mutational sites and that within each unit these are linearly arranged (26, 64 to 66). The fact that in general all mutant changes leading to defects in a specific biosynthetic reaction are found to lie within a single small segment of the genetic map is in agreement with the hypothesis that the total specificity of a given enzyme is determined by a single DNA segment.

In a number of instances in *Salmonella*, genes concerned with successive steps in the biosynthesis of required metabolite are found to be closely linked and to lie in a sequence that corresponds precisely with the sequence of steps in the biosynthetic pathway. Why this should be so is not clear. Jacob (16) has suggested that there might be a selective advantage in a system in which all steps in a sequence can be regulated together and that this may often be accomplished by an episomal repressor of some sort.

Hemoglobin.—In contrast to the above mentioned investigations on phage and *Salmonella* which demonstrate fine structure in the genetic material but not that of the presumed protein products of gene action, the hemoglobins of higher animals, especially of man, represent systems in which the fine structure of the product of gene action is more amenable to investigation than is the genetic material itself.

There are now known at least ten genetically differentiated molecular species of hemoglobin (67). Each differs in electrophoretic mobility from normal adult (A) hemoglobin. Normal A-hemoglobin molecules are made up of two pairs of polypeptide chains called α and β, the partners of which are identical, and four heme groups. As Ingram has shown (68), S hemoglobin differs from A in that valine replaces a particular glutamic acid unit in each of the β chains. In C hemoglobin, lysine is similarly substituted for this same glutamic acid unit of the β chains (69). The genes that differentiate S hemoglobin from A and C from A are allelic, each presumably differing from the normal counterpart in sequence of nucleotides.

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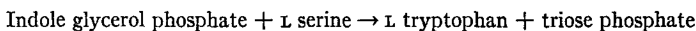
It is known that under mildly acidic conditions hemoglobin molecules reversibly dissociate to give two subunits of approximately equal size. Recently it has been shown in three separate ways by Itano and others (70 to 72) that this dissociation is asymmetric. The two α chains separate as one unit from the pair of β chains. Two of these proofs involve the formation of "hybrid" molecules, the α and β halves of which come from different original molecules. Thus *S* hemoglobin, which differs from *A* in the β chain, and *I* hemoglobin, which differs from *A* in the α chain (73), form two recombinant molecules when mixtures of them are dissociated and reassociated; one recombinant is identical with *A* hemoglobin and one has the defects of both *S* and *I* (72).

The quadripartite and dissociable nature of hemoglobin molecules raises several questions of genetic significance (67, 72, 74). Does a single continuous segment of DNA specify both α and β polypeptide chains? The answer seems to be no, as individuals heterozygous for two hemoglobin defects, *Ho*-2 and *S* (the first presumed to be in the α chain and the second in the β chain) who marry normal spouses, have children of three kinds with respect to their hemoglobin types (75). Their hemoglobins are: *A* plus *Ho*-2; *A* plus *S*; and *A* plus *Ho*-2 plus *S*. If the genes for the two defects were allelic, only the first two types would be expected. With two freely recombining genes, four types would be expected: those given above and, in addition, a class with only *A* hemoglobin. Absence of this class may well be attributable to small sample size, for only 15 offspring from marriages of the indicated type have so far been observed.

The fact that individuals heterozygous for genes responsible for defects in both the α and β chains have normal hemoglobin (75) presumably means that the α and β chains are separately synthesized and thereafter combined in the four combinations: $\alpha\alpha\beta\beta$, $\alpha\alpha\beta'\beta'$, $\alpha'\alpha'\beta\beta$, and $\alpha'\alpha'\beta'\beta'$ where α' and β' designate defective α and β polypeptide chains. The $\alpha'\alpha'\beta'\beta'$ type has not been identified electrophoretically in doubly heterozygous individuals, which as Itano points out (74) may mean that the changes in net charge produced by the two defects are equal and of opposite sign as in hemoglobins *C* and *I* (72). If this were so, $\alpha'\alpha'\beta'\beta'$ would not be different in mobility from $\alpha\alpha\beta\beta$.

Since in single-gene-pair heterozygotes such as those for *S* and *A* hemoglobins, only $\alpha\alpha\beta\beta$ and $\alpha\alpha\beta'\beta'$ molecules are present, never $\alpha\alpha\beta\beta'$ (67), it must be that the β chains are formed in pairs at the site of synthesis and thereafter remain associated. This must also be true for the α chains. This would further imply only one RNA template of a kind per microsome, for it is not easy to see how, otherwise, hybrid chain-pairs could be avoided.

Tryptophan synthetase.—The normal *in vivo* terminal reaction of tryptophan synthesis in microorganisms is believed to be:



Indole glycerol phosphate + L serine → L tryptophan + triose phosphate

catalyzed by the enzyme tryptophan synthetase. Many mutants of *Neurospora crassa* and *E. coli* in which this enzyme is inactive or altered have been

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investigated. Crawford & Yanofsky (76) have shown that in *E. coli* this enzyme is dissociable into two protein components *A* and B which separately have little activity in catalyzing the above reaction but which show restored activity when mixed. In investigations of the genetic, enzymatic, and immunological characteristics of this system (77), 13 mutant strains of *E. coli* have been classified into the following groups: three mutants in which both *A* and *B* components of the enzyme are inactive or absent and in which no protein immunologically related to either *A* or *B* is found; four in which no active component *A* is demonstrable, but in two of which a protein immunologically related to *A* is found; six in which no active component *B* is found but in three of which a protein related to *B* is demonstrable by immunological techniques. All mutants of the second two classes, i.e., those in which either *A* or *B* components are inactive, have active *B* and *A* components respectively and are capable of reverse mutation. They are therefore judged not to be the result of deletions of appreciable segments of genetic material, as is believed to be the case for the mutants of the first class.

Genetic mapping of the single-component mutants by the co-transduction technique reveals that the mutational sites involved in *A*-component mutants fall within a small region of the map and appear to be linearly arranged. The mutational sites of the *B* mutants are likewise all closely linked and they too seem to be linearly disposed. The two segments of genetic material corresponding to *A* and *B* mutants are adjacent to each other but not overlapping.

The agreement of these observations with the hypothesis that a linear gene code is responsible for the linear arrangement of amino acids in a polypeptide chain is obvious. The progress already made with this system is so encouraging that one can reasonably hope soon to see a comparison of positions of amino acid sequences in the protein components with the genetic map of mutational sites.

Adenylosuccinase.—The splitting of adenosine monophosphate succinate to form adenine and fumaric acid is catalyzed by the enzyme adenylosuccinase. The activity of this enzyme is readily measured *in vitro* down to a level of about 1 per cent of its normal activity. Its specificity for a second natural substrate, 5-amino-4-imidazole-(N-succinylo-carboxamide) ribotide, which is an earlier intermediate in adenine synthesis, is easily compared with that for adenosine monophosphate succinate. For these and other reasons the details of its genetical control have been investigated, especially in the laboratory of Giles (78, 79).

Of 123 mutants of *Neurospora crassa* of independent origins with specific requirements for adenine and lacking adenylosuccinase activity, all were found to be the result of changes in one small segment of chromosome. Many were found capable of back mutation, but in an extensive search by sensitive methods none was found subject to suppression by mutation at a separate locus. Intercrosses of different mutants give wild-type recombinants with a low frequency, but unfortunately there is so much sterility in such inter-

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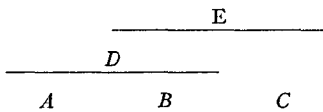
crosses that detailed genetic fine-structure studies of the chromosome region concerned have so far not been practicable.

Complementation.—The finding by Calef in 1956 (2) that some combinations of adenineless-9 mutants of *Aspergillus* presumed to be blocked in the same chemical reaction showed complementation in heterocaryons as evidenced by growth without adenine, whereas others did not, created much interest and considerable puzzlement among geneticists. How define a cistron in such a case? A similar situation was reported at about the same time by Giles and as additional examples began to appear, interest in the phenomenon increased rapidly. It was soon found that within groups of mutants concerned with a single chemical reaction, there are definite patterns of complementation.

Adenylosuccinase mutants of *Neurospora* will illustrate a part of the story. Woodward *et al.* (78) tested the 123 mutants mentioned above for complementation in various heterocaryon combinations. Many failed to show growth in any combination, but 51 gave complementation in at least one combination. When all combinations of complementing mutants, some of which involved secondary mutations derived from back-mutated primary mutants, were considered it was found that they could be represented by a so-called complementation map. A simple example of such a map is represented with a series of lines as follows:



Each single line represents a group of mutants within which complementation does not occur. Mutants in groups represented by non-overlapping lines do complement. Mapped in this way, the adenylosuccinase mutants reveal seven apparent cistrons.

What does it mean that all mutants can be put into a unique linear system of this kind? Comparison with a fine-structure map based on recombination cannot be easily made with the adenylosuccinase mutants because the crosses between mutants show much reduced fertility. That the two are closely correlated, however, is shown by the pantothenic less-2 locus of *Neurospora* and others for which both types of analysis have been made (79).

Heterocaryons carrying two complementing adenylosuccinase mutants grow without an exogenous supply of adenine and, as expected, produce active adenylosuccinase. Both growth rate and amount of enzyme activity are related to distance apart of the mutants on the complementation map. Heterocaryons between mutants in adjacent cistrons grow slowly and may show as little as 1 per cent of the enzyme activity of the normal strain from which the mutants were obtained. As the distance between mutants on the complementation map increases, growth of heterocaryons between them becomes progressively better and enzyme activity increases. But the latter seems never to exceed 25 per cent of normal activity significantly.

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What is the meaning of complementation itself? First, from the practical standpoint, it means that the definition of a cistron becomes difficult and at best arbitrary. In the adenylosuccinase case, it is obvious that if the criterion for complementation were enzyme activity and the assay used were not capable of detecting levels below 10 per cent, there would be fewer than seven cistrons. If it could detect less than 1 per cent there would probably be more. Much more important than the operational usefulness of the cistron is the question of the meaning of complementation in terms of gene-enzyme relations. It is clearly not confined to a few special cases for it has now been reported in *Aspergillus* (2), *Schizosaccharomyces pombe* (80), *E. coli* (81), and *Neurospora*. In the latter it has been demonstrated for at least eight genes but appears not to occur for several others (82, 83). Several possible explanations have been considered, viz: (*a*) compound enzymes, like tryptophan synthetase, with activity produced by reassociation of subunits; (*b*) switching templates in the transfer of information from DNA to RNA, i.e., a good RNA message copied from two defective DNA codes; (*c*) similar switching of templates in the final synthesis of protein; (*d*) enzyme activity dependent on formation of dimers or higher polymers, this being prevented if units are mutant and all alike but not if they are mutant and of two kinds.

Several lines of evidence bear on the question of which if any of these possible explanations is correct. Woodward (84) has recently succeeded in demonstrating complementation *in vitro* by preparing an extract in a special way from pairs of adenylosuccinase mutants of *Neurospora* which show complementation *in vivo*. This would seem to exclude template switching mechanisms in this particular case, for it does not seem likely these would continue to operate *in vitro*. Template switching at the DNA-RNA level is most unlikely in *Neurospora* in any event, for the DNA templates that would have to be involved are carried in separate nuclei.

Fincham (85) has found that some glutamic dehydrogenase enzymes formed by complementation are clearly different from the normal form of this enzyme. This seems also to be the case for adenylosuccinase complementation (84). This would exclude the hypothesis of randomly combining subunits, at least in its simplest form, for this would predict a normal enzyme. In addition the observations on adenylosuccinase suggest that this enzyme would have to be made up of at least seven non-identical subunits if the reassociation explanations were correct. This puts a considerable strain on the imagination. Fincham's finding would also appear to exclude a simple hypothesis of template switching for this too would be expected to give a normal enzyme.

Although there is relatively little direct evidence bearing on the fourth possibility, an hypothesis advanced by Allison (86) to explain the behavior of human haptoglobins (serum proteins—probably glycoproteins—that combine with hemoglobin) suggests a way in which enzyme complementation might occur through polymerization. The facts that prompted Allison to formulate this hypothesis are themselves of genetic interest in several respects. In brief, they are as follows: the alleles Hp^1, Hp^2 control the hapto-

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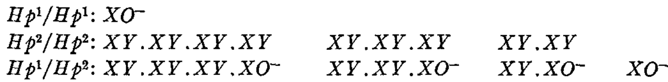
globins in question. Haptoglobins of the two homozygotes differ in charge and in the fact that in one they are homogeneous in molecular weight and in the other heterogeneous. The haptoglobins of the heterozygote are not a simple mixture of the two, there being present molecules of types not found in either homozygote. Allison's proposed explanation can be represented as follows:



where XO^- and XY represent monomers with one combining site or two. quantities X and Y are complementary combining sites, and the minus superscript indicates a charge difference. As Bearn & Franklin (87) and Allison (86) point out, it is conceivable that interactions of this kind might be the basis of the so-called hybrid substances in doves and rabbits that have puzzled immunogeneticists for so many years.

It is possible that an essentially similar interaction might account for enzyme formation by complementation. Assume the normal allele of a gene to specify the unit XY and two mutant forms, the units XO and OY. If dimerization were essential for enzymatic activity, the heterozygotes or heterocaryons would produce active enzyme in the form of $OY.XO$ dimers whereas homozygotes would not. A more complex but essentially similar scheme might account for situations like that of complementation in adenylosuccinase.

It is of interest that Soyama (88) reports animal glutamic dehydrogenase to be a polymer which normally has at least eight subunits per molecule, probably identical. The separate subunits seem to be active. If in two mutants subunits were inactivated in different ways, activity might be restored by co-polymerization.

Clearly, additional information is needed before it can be settled which mechanism or mechanisms are responsible for complementation.

Galactosidase.—From time to time there have been found gene-enzyme relations that have seemed quite irreconcilable with the hypothesis that the entire structural specificity of an enzyme is provided by a single gene. The lactose mutants of *E. coli* were an example. Recently Pardee, Jacob & Monod (89) have reinvestigated the genetics of β-galactosidase formation and activity in *E. coli* with the result that the lactose mutants now appear to support the hypothesis. They have found the "Lac" region to consist of three closely linked but physically separate and functionally different genes. One of these, the *z* gene or cistron, appears to carry the specifications for β-galactosidase structure.

Of 28 galactosidase-negative mutants investigated by the co-transduction technique, all were found to lie in the *z* region. Eight have been unambiguously mapped and found to represent as many separate linearly arranged sites within the *z* gene. Three mutants show certain as yet unanalyzed

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anomalous properties. Complementation does not occur in any combination tested. Some *z* mutants possess enzymatically inactive proteins immunologically related to galactosidase, and these differ among themselves. It seems clear that the primary function of the *z* gene is the determination of β-galactosidase structure.

Adjacent to the *z* locus is a gene *i* which determines whether β-galactosidase will behave as an inducible or constitutive enzyme. Its active allele is assumed to be essential to the synthesis of a "repressor" of β-galactosidase synthesis. In induction the repressor is antagonized. Inactive alleles of *i* block repressor synthesis with the result that *z-i* bacteria produce β-galactosidase without induction by galactosides.

The third component, the *y* gene, is presumed to be concerned with a galactoside-transport mechanism, called by Pardee *et al.* a permease. This may be either constitutive (i^-) or inducible (i^+); that is, the repressor controlled by i^+ represses both the galactosidase and the transport system.

Three of the "Lac" mutants that had previously been reported to lie at different positions in the chromosome were re-examined. Two were found to produce both galactosidase and galactoside-permease. A third was found by co-transduction to be in the *z-i-y* segment. Space does not allow a detailed description of all the ingenious means by which evidence was obtained for the above interpretation, nor does it permit more complete description of the system itself.

Suppressor genes.—In *Drosophila* and other organisms, suppressor genes have long been known. For example a recessive suppressor of vermilion in *Drosophila* restores ability of a genetically vermilion fly to synthesize brown eye-pigment. It has often been suggested that such suppressor genes may supplement or entirely replace faulty structural specifications resulting from mutation in the gene that originally contained them.

In investigations of suppressors of tryptophan synthetase mutants of *Neurospora*, Suskind & Kurek (90) have shown that quite simple alternative explanations may apply in at least some cases.

Many tryptophan synthetase mutants of *Neurospora* are known. All are alleles—or "heteroalleles" in the sense that they involve mutation at various sites within one gene. Some produce enzymatically inactive proteins immunologically related to tryptophan synthetase—i.e., cross-reacting proteins. Others do not. Suppressor genes in *Neurospora* are known for some but not all mutants that produce cross-reacting proteins; none are known for mutants of the other class. Suppressors are successful in restoring enzymatic activity only for certain mutants among those that produce cross-reacting proteins; that is, they are specific for certain alleles.

It has been found that enzyme activity can be restored to the cross-reacting protein of mutant 24 by separating it from an inorganic inhibitor. This inhibitor does not reduce the activity of normal enzyme. Hence it appears that mutant 24 has produced a qualitative change in the enzyme of such a nature that it is subject to complete inhibition by a normal com-

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ponent of the cell. The suppressor gene is evidently concerned with presence or availability of its inhibitor. In those respects so far studied, zinc duplicates the effects of the inhibitor. With this system it has been possible to determine that the suppressor gene does not supply specifications for enzyme structure. If it did, enzyme from suppressed mutant 24 should be inhibitor-resistant like normal enzyme. If not, enzyme from suppressed mutant 24 should be inhibitor-susceptible like that from the unsuppressed mutant. The latter was found to be the case in the *in vitro* response of partially purified inhibitor-free enzyme to natural inhibitor and to zinc. A similar situation was found for mutant 3 which also produces a metal-susceptible enzyme.

What new insight will be gained from further studies of suppressors can only be surmised. It will be of special interest to determine the nature of genetic suppression in tryptophan synthetase-less mutants of the type reported by Yanofsky & Crawford in *E. coli*, in which there appears to be no cross-reacting protein (77). It is already clear, however, that there is no compelling need to assume that the structural specifications for tryptophan synthetase are supplied by more than a single gene—or that this gene carries any other biologically useful specifications.

Tyrosinase.—Several years ago Markert & Owen (91) showed that mutants of *Glomerella* with reduced melanin are characterized by corresponding reductions in tyrosinase activity. Such mutant types were found to be of relatively frequent occurrence, and those apparently entirely devoid of tyrosinase activity were found to result from mutation at several loci. Does this mean that specifications for tyrosinase structure are derived from several genes? The investigations of Horowitz and collaborators (92) on the tyrosinase of *Neurospora* suggest that this is not the case, that the genetic control of tyrosinase may be basically no different from that of other enzymes.

Four alleles of a single gene at the *T*-locus have been found to be characterized by qualitatively distinguishable tyrosinases, suggesting that this is the gene responsible for structural specificity. Heterocaryons in which two such alleles are present produce what appear to be simple mixtures of the two enzymes.

Two other genes have been investigated in which mutation appears to abolish enzyme activity. In these it is found that enzyme production can be induced by any one of a number of aromatic amino acids and that when they are so induced, the enzyme produced is that specified by the allele present at the *T*-locus. These two genes are therefore probably concerned with the function of the enzyme, not with structure. As Horowitz *et al.* point out, tyrosinase is unusual in that it is not essential to the organism, at least for its vegetative multiplication and reproduction. The selective pressures against indirect inactivating mechanisms might therefore be relatively slight. Forms of the enzyme readily inhibitable in a variety of ways, as is the case for some mutant types of tryptophan synthetase enzymes, might therefore have become established as "normal".

Xanthine dehydrogenase in Drosophila.—Xanthine dehydrogenase activ-

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ity is absent in both maroon-like and rosy mutants of *Drosophila melanogaster* (93). Since these two eye-color mutants are differentiated from the wild type by mutant genes located in different chromosomes, it was not unreasonable to assume that both may contribute information essential for the synthesis of enzyme. However, with the recent discovery by Glassman & Mitchell (94) that maroon-like mutants show a maternal effect explainable on the assumption that the normal allele of the mutant gene is essential for a substance *X*, necessary for enzyme activity and capable of being carried to a subsequent generation through the egg, it seems more probable that this gene is concerned with the production of a cofactor of some sort rather than in supplying information for the protein component of the enzyme.

Virus-directed protein synthesis.—Viruses, especially phages, have several important advantages for the investigation of the relation between genetic DNA and specific proteins (23). High-resolution genetic analysis is possible and nucleic acid is readily available for direct physical and chemical investigation. The protein coats, obviously synthesized under the direction of phage DNA, are likewise easily available in a relatively pure state.

Already, encouraging progress has been made in identifying several enzymes that appear to be made in infected host cells under the influence of phage DNA. Flaks & Cohen (95) have shown that bacteria infected with T2 phage have an enzyme that hydroxymethylates deoxycytidine 5-phosphate, the product being a precursor of phage DNA not normally synthesized by bacteria. Kornberg *et al.* (96) have now isolated and identified three enzymes found in T2-infected bacteria but not detected in normal bacteria. One of these phosphorylates hydroxymethyldeoxycytidine 5-phosphate to the triphosphate. A second removes the terminal pyrophosphate from deoxycytidine triphosphate. The third catalyzes the transfer of glucose from uridine diphosphate glucose to phage DNA containing hydroxymethylcytosine. These four enzymes, synthesized in response to T2 DNA within a few minutes of its injection, go a long way toward explaining the presence of hydroxymethylcytosine in T2 DNA, and its partial glucosylation. Whether these constitute the whole of the "early" protein that must be synthesized, along with RNA, in a T2-infected cell before phage DNA can be replicated is not known (22, 23, 97, 98).

Smaller phages, like $\phi X174$ with only 5500 nucleotides per phage unit of DNA, offer even greater hopes for identifying chemically defined DNA with corresponding proteins. Considering that a substantial fraction of the DNA must be required to specify the protein coat, it seems doubtful that DNA in such a virus is sufficient to determine the structure of more than three or four enzymes.

Mutation

Only a few special aspects of this large general subject are considered here.

Radiation-induced mutation.—Of the many excellent reviews of the subject of radiation-induced mutation, two have appeared recently, one by

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Muller (99) and one by the United Nations Scientific Committee on the Effects of Atomic Radiation (100). It should be pointed out that Russell *et al.* (101, 102) have recently reported a most significant new fact, namely, that chronically administered ionizing radiation is less effective per r unit by a factor of something like four in producing mutations in spermatogonia or oöcytes of mice than is an equal amount given in a single acute dose. This difference is not found for mature spermatozoa of either mice or *Drosophila*. The new findings have important implications as to the mechanisms of radiation-induced mutations and, of course, bear on the matter of hazards to man of long-time exposure to low levels of ionizing radiation.

Mutation (defined in a broad sense to include chromosome breakage) induced by P^{32} incorporated in phage particles, bacteria, and cells of other organisms has proved to be a useful tool in the investigation of several processes of biological importance. Stent and Fuerst have provided a most useful summary of the subject (28). It seems highly probable that P^{32} incorporated in DNA produces its direct genetic effects primarily through nuclear recoil and transmutation. In both phages and bacteria the lethal effects probably result from simultaneous breakage of the two chains of the DNA molecule by properly oriented nuclear recoils, for the efficiency of P^{32} disintegrations in inactivating DNA from T phages or bacteria is about 0.1, the same as that for breaking double DNA molecules *in vitro*, whereas for phages with single stranded DNA, the efficiency is approximately 1.0.

The radioisotope P^{32} is effective in inactivating phages before or just after injection. But within a few minutes after injection, phage DNA is somehow stabilized against P^{32} inactivation. This stabilization appears not to be caused by the formation of P^{32}-free replica molecules or by the ability to reconstruct good DNA units from broken ones, but rather by some process involving protein synthesis. Protein that stabilizes one phage seems not to stabilize a genetically different phage introduced later, possibly because the protein required is specific.

Stent (21) has proposed a mechanism of DNA replication involving transfer of genetic information from double DNA molecules to RNA-protein complexes which in turn direct the synthesis of new double DNA molecules. The postulated RNA-protein templates would contain no P^{32} under the conditions of most experiments, and even under conditions in which they do, the protein could conceivably confer stability.

Since it is not at all obvious how the Stent mechanism would give the "hybrid" DNA molecules observed by Meselson & Stahl (29, 30) or operate in the Kornberg system (31, 32) in which specific DNA appears to be synthesized in the absence of either protein or RNA specifically related to DNA, it seems clear that alternative explanations for stabilization of DNA against P^{32} breakage should be explored. Possibly protein serves directly to stabilize DNA, forming deoxyribonucleoproteins of the kind Zubay & Doty have isolated from chromosomes (47).

The P^{32}-decay method has also been used most ingeniously in investigating bacterial conjugation. If P^{32}-labeled donor cells, with appropriate

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genetic markers, held at −196°C., are thawed after various intervals and then allowed to conjugate, it is found that the farther removed from the "head" of the entering chromosome a genetic marker, the more rapidly is its probability of entering the recipient reduced, i.e. the more likely a break ahead of it. The quantitative agreement of this reduction with map positions as determined by time of entrance is remarkably close.

Doudney & Haas (103) have recently extended the earlier finding of E. M. Witken that frequency of recoverable bacterial mutants induced by ultraviolet is strongly correlated with postirradiation protein synthesis. The facts, which unfortunately cannot be restated here, suggest to these workers that mutation occurs through the alteration of cytidine and uridine precursors of RNA, that these are "stabilized" as amino acid RNA complexes and then "fixed" irreversibly as mutations in ribonucleoproteins which serve as templates for DNA double helices in the manner proposed by Stent for DNA synthesis via RNA-protein templates. As indicated above, it is not immediately evident how the Stent hypothesis and the new evidence on DNA synthesis are to be reconciled. If they cannot be, the remarkable interrelations demonstrated by Doudney & Haas must of course be interpreted in some other way, as Witkin (104) has pointed out.

Chemical mutagenesis.—Since the first clear demonstrations in the period 1943 to 1946 that mutations could be induced by various chemical agents (105), hundreds of compounds, inorganic and organic, have been shown to be mutagenic or antimutagenic (105 to 107). The mechanisms seem clearly to be several, some direct, others indirect.

One of the very important advances in this area is the recent discovery by Schuster & Schramm (108) that nitrite produces mutations in pure RNA of tobacco mosaic virus by oxidation of amino groups to hydroxyl groups. This oxidation converts cytosine to uracil, adenine to hypoxanthine, and guanine to xanthine. It appears that oxidation of any one of about 3000 of the 6000 nucleotides present in the RNA of a single virus particle leads to inactivation. The effect is the same whether RNA is treated in intact viruses or after removal of protein coats.

In addition to lethal mutations, some changes leave the RNA infectious and viable but altered in its effect on the host. Thus Mundry & Gierer (109) have found that nitrite increases by a factor of 20 mutation to types that give necrotic instead of chlorotic local lesions in a particular test. This is by treatment that inactivates only about half the RNA units. It is estimated that an oxidation of any of about 180 nucleotides in the 6000 total leads to such a "necrotic" mutation. So many other mutations are detected at this level of oxidation that it can be inferred that the majority of treated RNA units are genetically modified.

It is tempting to speculate that oxidations leading to replacement of cytosine with uracil will often give viable mutations, since both of these pyrimidines normally occur in RNA. Conversion of a natural to an unnatural base might, on the other hand, be expected to be lethal.

Base-analogue mutations.—Following the demonstration that the pri-

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mary genetic material of some viruses and of cellular forms is DNA, it was natural to expect that analogues of nucleotides (base-analogues) might be incorporated during DNA replication and thus lead to genetic change. This has indeed been found to be the case.

An especially illuminating investigation of such mutations has been made by Freese (110) using phage T4 and the Benzer rII technique. It was already known that spontaneous mutations differ in distribution of sites involved from those induced by 5-bromouracil and proflavin and that the latter agents differ among themselves in this regard. Freese has found that most mutations induced by the base-analogues 2-aminopurine and 5-bromodeoxyuridine are reversible to the standard wild type by further exposure to base-analogues (296 out of 300 tested) but that those induced by proflavin are not induced to revert in this way despite the fact that many revert spontaneously. To account for these and related findings, Freese postulates two kinds of single base pair substitutions, viz:

(*a*) Adenine-Thymine $\rightleftarrows$ Guanine-Cytosine

i.e. replacement of purine with purine and pyrimidine with pyrimidine. Base-analogues should induce changes of this kind since presumably purine analogue replaces only purine, and pyrimidine analogue only pyrimidine. In following DNA replications, replacements of analogue by the natural nucleotides would be expected either to restore the original condition or to substitute base-pairs as above.

(*b*)

$$\begin{array}{ccc} \text{Adenine-Thymine} & \rightleftarrows & \text{Thymine-Adenine} \\ \downarrow\uparrow & & \downarrow\uparrow \\ \text{Cytosine-Guanine} & \rightleftarrows & \text{Guanine-Cytosine} \end{array}$$

i.e., replacement of purine with pyrimidine and vice versa through mistakes in incorporation of nucleotides during replication. There is no reason to believe base-analogues would increase this type of mistake or reverse it once it has resulted in base-pair transversion in a daughter DNA double helix. About 10 per cent of spontaneous mutations are reversible with base analogues, suggesting that this proportion is of the first class. As Freese (110) points out, this hypothesis is testable in several ways and no doubt such tests will soon be made.

Kaudewitz (111) has reported a curious delay in expression of ultraviolet-induced mutations in *Salmonella* which he interprets as detachment of bits of genetic material transmitted linearly for several generations with final reincorporation.

Paramutation in maize.—Mendel's rule that genes segregate from heterozygotes uncontaminated by association with alternate forms is of course violated by intragenic recombination. It now appears that it may fail in another way as well. Brink and his collaborators (112) have discovered that the R^r gene concerned with endosperm pigment in maize is invariably modified by association with the R^{st} (stippled) or R^{mb} (marbled) allele in

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heterozygotes. This change, detected as a reduction in pigmentation, is reversed slowly but apparently not completely over a period of generations after removal of the R allele from association with R^{st} or R^{mb}.

While several characteristics of paramutation at the R locus are known, for example that the R^{st} is not itself detectably altered during the process and that the effect in trisomics of the genotype R^{st}, $R^r r^g$ is the same as in $R^{st}R^r$ diploids, the latter fact showing that close meiotic pairing is not essential, the basic nature of the process is not yet understood.

Coe (113) has found a somewhat similar case involving the B (plant color) locus in maize. Here a B' allele appears to have become paramutagenic toward a standard B allele, always converting it to a B' which is likewise paramutagenic. Hagemann (114) reports what may be a similar phenomenon in the tomato.

The Coding Problem

On the hypothesis that DNA serves to specify sequence of units in RNA, which in turn specifies amino acid sequence in proteins, several linear coding systems have been explored. One of these (115 to 118) assumes that amino acids are specified by non-overlapping triplets of base-pairs (or single nucleotides in one chain) of DNA, of which 20 are possible with four nucleotides. If provision is made for reading the DNA molecule in one direction only, so-called comma-free transposable codes result, in any one of which there are only ten usable triplets (117). If quadruplets ("four letter words") are similarly used, there are 27 good "words" per code, clearly enough to encode all the amino acids that occur in proteins (117).

Sueoka *et al.* (119) and Sinsheimer (120) have pointed out a possible difficulty with such four-symbol coding systems: the guanine plus cytosine/adenine plus thymine ratio varies from less than 0.4 to more than 0.6 in the DNA of four bacteria Sueoka *et al.* and others have investigated, but within a single species the molecules are remarkably homogeneous in this regard (119). RNA in the same species varies in composition in a corresponding way but to a much smaller degree. On the basis of the widely held belief that the majority of the proteins of the bacterial species investigated are alike or closely similar, the mean DNA composition should show correspondingly little variation from species to species. What the observed variation means is therefore not clear. Perhaps the proteins vary more widely than is commonly believed. If not, possibly only a part of the DNA is genetically useful. This would have to be less than 10 per cent if the proteins are essentially alike. One of several alternative possibilities is that the coding system may involve sequence of amino and keto groups in the 6-positions of purines and pyrimidines; that is, a two-symbol code (119, 120). How such a code might work in detail has not yet been made clear.

Investigation of the properties of various possible coding systems is valuable, for each of them has something to say about possible sequences

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of amino acids and the kinds of substitutions of amino acids that can be made by specified types of changes in the code (121, 122).

The investigations of Ingram and co-workers (68, 69, 73) on amino acid sequence in hemoglobins are a beginning in this type of analysis. It can be anticipated that progress will be rapid. Perhaps it will come in part through more knowledge of the nucleotide sequences of the soluble cytoplasmic RNA's now assumed by some investigators to bear specific relations to individual amino acids. It is not out of the question that the nature of the coding system may be discovered without resort to the discouraging task of determining by chemical methods the nucleotide sequences in either RNA or DNA.

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Other Areas of Genetics

In addition to those already mentioned, there have been other significant advances in genetics within the past two or three years which cannot be reviewed in any detail. There continues to be keen interest in mechanisms of recombination in phages (123, 124); in integration of DNA during bacterial conjugation (13), transformation (125, 126), and transduction (66); in crossing over, so-called conversion, and other phenomena that occur during meiosis (127 to 133); and in McClintock-type controllers (134).

It is now quite clear that the normal chromosome number in the somatic cells of man is 46 (135, 136) instead of 48 as believed for so many years, although it remains a possibility that in the germ line of some normal individuals there may be 47 or 48 (137). Three congenital diseases of man have now been shown to involve chromosome imbalance. Mongolism is associated with presence in somatic cells of a small extra autosome (138 to 140); Klinefelter's disease is the result of sex-chromosome imbalance, affected individuals being sterile males *XXY* in constitution (140, 141). Turner's disease affects *XO* females incompletely developed sexually (142, 143). The phenotypes of *XXY* and *XO* individuals indicate a positive role of the *Y* chromosome in sex determination. Interestingly, a similar role of the *Y* chromosome of the mouse has recently been reported (144, 145). Perhaps this is general for mammals in contrast to the situation in *Drosophila*.

The possibility of using bone-marrow transplants in the therapy of severe radiation injury has greatly increased the interest of the medical profession in immunogenetics. There are several excellent reviews of genetic aspects of the general subject of tolerance to tissue transplants, among them those by Owen (146, 147), Brent & Medawar (148), and Billingham (149). More general aspects of immunogenetics are considered by Ebert (150) and by Schultz (151).

Partly because of increased interest in the genetic hazards to man of high-energy radiations and partly because of increased recognition of the importance of genetic disease (48), attempts are now being made to develop new techniques of investigating human genetics. Among the most promising of these is the use of cell populations either *in vivo* or in tissue culture (2, 152 to 154).

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In August of 1958 the Oak Ridge National Laboratory sponsored a symposium on the genetic aspects of somatic cell variation. The papers presented, plus the discussion provoked by them, cover such subjects as nuclear changes in somatic cells, cytoplasmic factors, antigen-antibody interrelations, the immunogenetics of transplantable tumors, cytology of tumors, and genetic studies of human cells in tissue culture; and they are a convenient and valuable summary of these important areas (155).

Markert & Møller (156) have confirmed and extended earlier work on animal enzymes showing that these may exist in multiple forms called "isozymes". Patterns of isozymes vary not only from one tissue to another but also during development of a single tissue and from species to species. Polymerization of the kind postulated by Allison (86) for human haptoglobins is one of several explanations considered for the observed heterogeneity. An obviously important question from the standpoint of development is whether such enzyme modifications are a primary cause of differentiation or a result of it.

The nuclear transplantation technique of Briggs & King (157) provides a most useful method of investigating certain aspects of development of multicellular organisms, especially nuclear-cytoplasmic interactions. Since it has long been obvious from classical genetics that the nucleus and cytoplasm must interact in a mutually compatible way, it is not surprising that the nucleus of a frog cell transferred to an egg cell of a different species leads to abnormalities in development. It could not have been predicted, however, whether such a sojourn of nucleus in a foreign cytoplasm would or would not do it irreversible harm. The preliminary results of such heterotransplantation experiments in frogs by Fischberg *et al.* (158) and by Moore (159) show that the nucleus is in fact adversely affected, for if after a number of cell divisions it is returned to an egg of its own species, development is invariably abnormal. In one set of experiments (158) some recovery seems to take place during the course of repeated back-transplants to eggs of the same species. That a nucleus should undergo irreversible or incompletely reversible damage under such conditions raises the question of whether nuclear changes that have been demonstrated to take place during normal development (157) are a secondary result of differentiation or a primary cause of it.

Two new approaches to the general problem of interactions between cytoplasm and nucleus have recently been reported, both in fungi (160, 161). Several aspects of this large and important general subject have been reviewed by Hämmerling (162); Catcheside *et al.* (163); Ephrussi (164); L'Heritier (165); and Nanney (166).

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