steroid dosage. This has, unfortunately, not been our experience with many other cases treated with 6-mercaptopurine. In any event, on the basis of the results obtained with massive corticosteroid therapy as contrasted with antimetabolites, we have concluded that this represents the method of choice in the therapy of acute leukemia in adults, particularly in patients between the ages of thirty and sixty. In our hands, the results with prednisone therapy as the sole medication have yielded more frequent and more sustained remissions than 6-mercaptopurine in adults, and without injury to the cells of the bone marrow, so commonly noted with antimetabolite therapy.

Summary

A case of acute leukemia in an adult, with a seven-year remission after treatment with massive doses of corticosteroids, is reported, together with some remarks on the relative value of large-dosage corticosteroid therapy as compared with antimetabolites in such cases.

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MEDICAL PROGRESS

THE EXPRESSION OF GENETIC INFORMATION*

John W. Littlefield, M.D.†

BOSTON

It has become widely accepted that within a cell genetic information is coded in the sequence of bases in deoxyribonucleic acid (DNA), that DNA can direct the synthesis both of itself and of ribonucleic acid (RNA) with an analogous base sequence and that the base sequence in RNA can be translated into the sequence of amino acids in a protein. The amino acid sequence in a protein molecule determines its function; the kinds and amounts of proteins in a cell determine what kind of cell it is. Since each cell contains many different proteins there are many different RNA templates, each representing the genetic information in a certain part of the DNA.

To spell out this theme in more detail, this review outlines briefly what is now known about the structure and synthesis of DNA, RNA and protein, about the nature of the code in which certain bases stand for each amino acid and about the ways in which genetic information may be modified and its expression regulated. This review does not attempt to cover completely or in depth such a diverse and active area of biologic research, but to orient in this area someone outside it. The limited literature cited stresses recent reviews, in addition to selected old and new papers that seem of special importance.

The greater part of the material to be covered derives from studies with bacteria, and an attempt has been made to delineate the past contributions and present status of work with higher organisms. It is disconcerting to note that conclusions drawn from studies with bacteria have frequently failed to apply in mammalian systems. It is sobering to realize that the unraveling of those complex biologic processes peculiar to multicellular organisms has just begun.

DNA Synthesis

Abundant evidence exists that DNA (or RNA in certain plant and animal viruses) is the hereditary genetic material throughout nature.[1] In this role it has two functions: to direct the formation of proteins and to duplicate itself before each cell division. Its mechanism of duplication will be considered in this section.

DNA is a very long helical molecule containing two intertwined strands. Each strand is a polymer of nucleotide units in which the 5′ phosphate of one nucleotide is bonded to the 3′ hydroxyl group of the deoxyribose of the adjacent nucleotide (Fig. 1). The nitrogenous base of each nucleotide (usually adenine, guanine, cytosine or thymine) forms hydrogen bonds with a base in the other strand of the DNA molecule, and these two hydrogen-bonded bases constitute a base pair. Adenine forms hydrogen bonds only with thymine, and vice versa, and guanine only with cytosine. For this reason the sequence of bases along each

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BASE = PURINE (Adenine, Guanine) or PYRIMIDINE (Thymine, Cytosine)

BASE + DEOXYRIBOSE = DEOXYRIBONUCLEOSIDE (Deoxyadenosine)

BASE + DEOXYRIBOSE + PHOSPHATE =
DEOXYRIBONUCLEOSIDE MONOPHOSPHATE
(Deoxyadenosine monophosphate or deoxyadenylic acid)

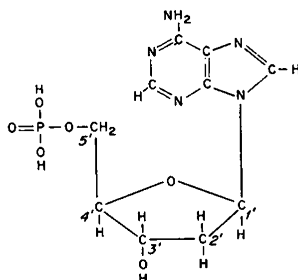

FIGURE 1. *Terminology.*

strand is complementary to that in the other strand. It is important that the sequence itself is under no restriction. The DNA molecule is accompanied by proteins containing a high proportion of amino acids with positively charged side chains, which are attracted by the negatively charged phosphate groups of the DNA. When this structure was first proposed in 1953 by Watson and Crick[2] it was suggested that precise duplication of DNA might be accomplished if the two strands separated during duplication, and on each was built a new complementary strand. Such a mechanism, formally termed semiconservative duplication, contrasts with a conservative scheme, in which the two parental strands remain together, and with a dispersive or fragmentative scheme, in which there is mixing of new and parental pieces in the daughter DNA molecule. Most subsequent studies, with bacteria or other organisms and heavy or radioactive isotopes to distinguish new from old DNA molecules, have confirmed the semiconservative mechanism of duplication originally proposed.[3] Recently, such studies have been extended to mammalian cells.

Experimental attack on the detailed mechanism of DNA synthesis really began with the work of Kornberg[4] and his co-workers, who in 1958 extracted an enzyme called "DNA polymerase" from cultures of *Escherichia coli.* This enzyme catalyzed the synthesis of DNA when supplied with a small amount of "priming" DNA, which seemed to serve as a pattern or template, magnesium ions and the deoxyribonucleotide triphosphates of the four bases. A deoxyribonucleoside triphosphate contains two phosphates attached to that present in a monophosphate (Fig. 1), and both these additional phosphates were split off during the polymerization. Several workers have found similar polymerase enzymes in rapidly growing mammalian tissues, and both the bacterial and the mammalian enzymes have been purified to some extent to free them of contaminating activity.

Current interest centers on the role of the DNA primer in the reaction and the exact mechanism of synthesis.[5] It was first shown that a DNA primer containing a certain ratio of adenine and thymine to guanine and cytosine directed the synthesis of new DNA with the same ratio. Subsequently, it was demonstrated that the sequence of bases in the newly synthesized DNA is the same as that in the primer, and that the predicted complementarity between the two strands does indeed exist.[6] Recent work indicates that the primer is incorporated into the product of the reaction. With some of the polymerase preparations so far examined, a single strand of DNA, which occurs on occasion in nature,[7] or can be produced by heat treatment of double-stranded DNA,[8] is a better primer than the intact double-stranded molecule. These findings are in accord with the original proposal that the double helix separates during duplication (Cavalieri[9] has advanced a dissenting opinion). For various reasons it has seemed unlikely that the entire double helix is split before duplication, but rather that it separates as it duplicates. It is uncertain whether or not duplication of the DNA primer begins at an end of one strand, as has usually been proposed, and whether the new nucleotides form covalent or hydrogen bonds with the primer strand.

It is difficult to envisage the orderly duplication of many DNA molecules organized into a chromosome.[10] There is too little knowledge of this structure, which includes RNA and probably several proteins, as well as DNA, in multiple strands. It is not clear, for example, just how long the DNA molecules in a chromosome are. With increasing awareness of the fragility of DNA have come larger estimates of its molecular weight.[11] Yet it is hard to picture how a molecule of great length could be maneuvered and duplicated without errors. Gentler extraction methods may provide a DNA molecule of apparently great length but in reality composed of many molecules, synthesized separately and then linked together. In any case it is known that chromosomes duplicate in a semiconservative manner like their component DNA molecules. After one duplication in the presence of a radioactive DNA precursor all the daughter chromosomes are labeled. Then, if the precursor is removed, the labeled chromosomes remain intact, except for reciprocal exchanges, during subsequent duplications.[12] These studies, which were carried out with plant cells, have now been repeated with mammalian cells in culture. Other recent studies with mammalian cells indicate that certain chromosomes are duplicated earlier than others and that duplication may start in certain areas, not necessarily the end, of each chromosome.

RNA SYNTHESIS

In recent years various types of RNA have been recognized, each with a different role in the transfer

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of information between DNA and protein. In contrast to DNA, for which almost all studies indicate synthesis and function to occur in the nucleus, at least part of the cellular RNA is synthesized in the nucleus but migrates to the cytoplasm. Three general categories are now recognized: ribosomal RNA, constituting the bulk of the cellular RNA; transfer RNA; and the newcomer, messenger RNA. More types will doubtless be distinguished, especially in the nucleus.

Like DNA, RNA is a linear polymer of nucleotides, linked by phosphate bonds. In RNA the 5-carbon sugar in each nucleotide is ribose, with a 2′ hydroxyl group which is not present in deoxyribose and which makes RNA susceptible to hydrolysis by alkali or certain enzymes. Very little thymine occurs in RNA, its place being taken by uracil. Several unusual bases are present in transfer RNA. RNA molecules are variable in length, though all shorter than DNA, and to a variable degree double-stranded.

The first type of RNA to be specifically implicated in protein synthesis, almost ten years ago, was that occurring in the ribonucleoprotein particles or "ribosomes."[13] At that time it was shown that the polymerization of amino acids into protein occurs on these particles, but the details of this process remain unclear (as discussed below). Ribosomes contain about equal amounts of RNA and protein, including structural proteins and certain enzymes as well as the protein being synthesized. They are unstable in low concentrations of magnesium, breaking down into progressively smaller inactive units. Ribosomes are present throughout nature; in mammalian cells they occur both in the nucleus and in the cytoplasm, where they may be free or bound to the lipoprotein membranes of the endoplasmic reticulum. The number of bound particles is high in secreting tissue such as liver and pancreas. In bacteria the number of ribosomes reflects immediately the growth rate.

The second type of RNA to be implicated in protein synthesis, about five years ago, was "soluble," or "transfer," RNA,[14] which is relatively small in size and acts as a carrier of activated amino acids to the ribosomes (as discussed below). It is nonparticulate in terms of the usual centrifugal forces employed in the fractionation of cells, and present in the nucleus and cytoplasm. For each of the twenty-odd amino acids there is at least one type of transfer RNA. All transfer RNA molecules terminate at one end with the base sequence -cytosine-cytosine-adenine; the 2′ or 3′ hydroxyl group of the ribose attached to the terminal adenine is the site at which an amino acid is bound. Recent studies suggest the transfer RNA molecule to be a single strand, bent back on itself like a hairpin.[15] Most of its bases form hydrogen bonds; those at the bend cannot, and might be the bases that represent an amino acid. A number of laboratories are engaged in separating the types of transfer RNA to locate and determine these specific base sequences.

It has seemed likely that transfer RNA interacts with ribosomal RNA by base pairing between the specific base sequence on the transfer RNA and a complementary sequence in ribosomal RNA. Studies in the past few years suggest the interposition of a third type of RNA, called "messenger RNA," in this process, but definitive interpretation of this work is not yet possible. It is convenient to distinguish experiments with bacterial systems, mammalian systems and synthetic RNA preparations.

When a bacterium is infected with a bacterial virus, or bacteriophage, there is a rapid shift in the types of protein made by the cell. This shift is preceded by the production, not of new ribosomal particles, but of a small amount of RNA that has a base composition analogous to that of the DNA of the bacteriophage. This RNA becomes associated with the ribosomes, and in other experiments has been shown to enhance and prolong the activity of ribosomes in cell-free extracts. It was postulated that such RNA acts like a messenger,[16-18] conveying information from DNA to instruct the ribosomes as to what kind of protein to synthesize. At first it was suggested that messenger RNA persisted on the ribosome only long enough to direct the synthesis of one protein molecule; recent studies indicate that it directs the synthesis of more than one.[19] It becomes difficult to distinguish experimentally messenger RNA from the precursors of ribosomal RNA. Nonetheless, the evidence favors a distinction between these two types of RNA in bacteria.

It has been known for some time that in the nucleus of the mammalian cell, there is an RNA fraction synthesized more rapidly than cytoplasmic RNA. Various experiments, mostly radioautographic, have suggested that this RNA migrates from nucleus to cytoplasm, but the quantitative aspects of these experiments have recently been questioned,[20] and the problem is under belated but intensive reinvestigation. Recent studies indicate that the rapidly synthesized nuclear RNA is relatively large and heterogeneous and that most of it is broken down in the nucleus and does not reach the cytoplasm.[20,21] Reticulocytes that lack a nucleus must synthesize hemoglobin without a constant supply of nuclear RNA. It now seems likely that a situation strictly similar to that in bacteria does not occur. If new ribosomes are made in the nucleus, a messengerlike RNA might be attached before they leave. A distinction between these two types of RNA may be hard to make in mammalian cells.

Another aspect of the problem stems from the exciting discovery by Nirenberg and Matthaei[22] in 1961 that under certain conditions "polyuridylic acid," a synthetic RNA containing only one type of base (uracil), directed the formation of a proteinlike polymer of phenylalanine ("polyphenylalanine"). They used an enzyme previously discovered by Ochoa[23] to make the polyuridylic acid. This was added to a cell-free extract containing ribosomes, transfer RNA and soluble enzymes that has been used extensively in studies on protein synthesis. In subsequent studies the

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groups of Nirenberg and Ochoa have used synthetic RNA preparations of different base compositions; these studies have suggested which bases in RNA represent each amino acid (as discussed below). The synthetic RNA preparations seem to be functioning as postulated for messenger RNA; as a potential model it is interesting to study the mechanism of their interaction with ribosomes. Part of the polynucleotide is broken down, but some complexes with some of the ribosomes. This aggregate, which might be a linear array of ribosomes along the polynucleotide molecule, is active in amino acid incorporation. The relation of transfer RNA to this aggregate should soon be clarified.

Thus, there is reason to believe that in bacteria, active ribosomes contain two interacting types of RNA, distinguishable by rate of labeling and base composition, and that the association between synthetic RNA and ribosomes may be a useful model of this interaction. The same thing cannot be said for mammalian cells at this moment.

The sites and mechanisms of synthesis of the various types of RNA are poorly understood. As indicated above, messenger RNA is probably formed on a DNA template, but it is not clear if this is also true for ribosomal and for transfer RNA. Naturally occurring complexes of DNA and RNA have been described, and under certain conditions it appears that each of the three types of RNA can be made to attach to specific regions of DNA.[24] It is known that RNA-synthesizing enzymes that do not require DNA exist. In recent years these have been overshadowed by the discovery in bacterial, plant and mammalian cells of enzymes that do require DNA to form RNA.[25,26] In plant cells these enzymes are closely associated with the chromosome.[27] The RNA produced has the base sequence (substituting uracil for thymine) of the DNA primer.

Protein Synthesis

Proteins are linear polymers of the twenty-odd amino acids, joined by peptide bonds, twisted into a helix and, in the case of the globular proteins, the whole folded upon itself (Fig. 2).[28,29] As in a nucleic acid, it is the sequence of amino acids — or, more strictly speaking, of the laterally projecting side chains of the amino acids — that distinguishes one protein from another. Certain side chains bond with others to determine the three-dimensional structure of the molecule; groups of other side chains determine enzymatic or immunologic activity. For a few proteins the entire amino acid sequence is known, and these enzymatically active groups have been tentatively identified.[30,31] In ways to be discovered the proteins of the cell wall appear to guide the interaction of one cell with another.[32,33]

The initial reactions in protein synthesis were clarified several years ago,[34,35] especially from studies with cell-free extracts of mammalian cells. It was established that in the presence of adenosine triphosphate,

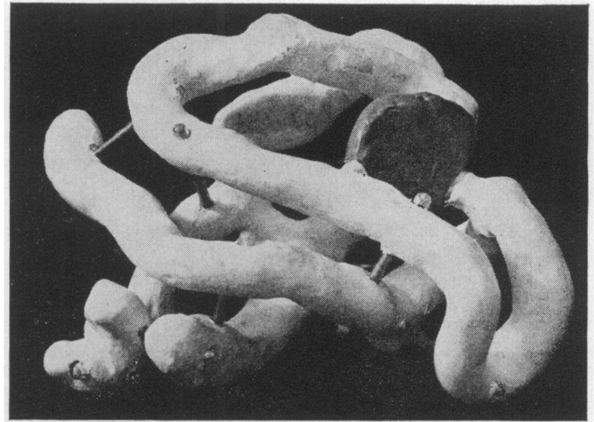

Figure 2. *Model of the Configuration of a Myoglobin Molecule (Reproduced from Kendrew[28] with the Permission of the Publishers).*

The heme group is at the upper right. Considerably more detailed knowledge of this structure is now available.

each amino acid is activated by a specific enzyme, which forms an enzyme-bound adenosine monophosphate-amino acid complex. Then the amino acid is transferred by the same enzyme to the 2′ or 3′ hydroxyl of the ribose attached to the terminal adenine of a transfer RNA molecule specific for this type of amino acid. Thus, the activating enzyme must distinguish between the base sequences of various transfer RNA molecules. The transfer RNA-amino acid complex is believed to attach to a site on the ribosome corresponding to that amino acid through the formation of hydrogen bonds between complementary base sequences on the transfer RNA and ribosomal or messenger RNA, as discussed above.[36] In this way the amino acids required for the protein under construction may be aligned on the ribosome in the desired sequence. After all the transfer RNA-amino acid molecules are aligned (or perhaps as each is attached) the amino acids are polymerized, and the transfer RNA molecules probably released for reutilization. The new protein molecule separates from the ribosomes, and its subsequent folding is probably automatic.

What happens to a new protein molecule after its polymerization depends upon its type. Soluble cell enzymes and other proteins, such as hemoglobin, are released into the cytoplasm. Proteins of internal or cell-wall structures must be either synthesized *in situ* or moved to places where they will aggregate. Proteins to be secreted follow yet another pathway — for example, pancreatic enzymes and liver albumin do not appear free in the cytoplasm. In the pancreas such proteins pass from the ribosomes across the adjacent lipoprotein membranes into the lumen of the endoplasmic reticulum and are moved first to the Golgi region and then in vacuoles to the cell surface.[37]

Coding

If the sequence of bases in an area of DNA determines via RNA the sequence of amino acids in a pro-

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tein, what is the base sequence for each amino acid? In the past decade several attempts have been made to derive from fragmentary data a scheme of how many, which and what order of bases in DNA represents each amino acid.[38] In the past year experimental confirmation that such a code exists and details about its nature have been obtained.

Of great significance was the discovery that polyuridylic acid directed the formation of polyphenylalanine in a cell-free extract from *Esch. coli,* as mentioned above.[22] Subsequently, synthetic RNA preparations containing various proportions of adenine, guanine, cytosine and uracil have been tested to determine which amino acids were polymerized in their presence.[22,39] These studies have given a tentative code for the twenty amino acids (Table 1).

Several features of this code deserve comment. As predicted in the past it seems likely that three bases, a triplet, constitute the code for one amino acid, although two may be adequate. In no case is the order of the three bases known. There is probably degeneracy in the code — that is, certain amino acids, such as leucine,[40] have more than one triplet. In reading the code one must be assured in some way that the adjacent bases of two triplets will not be mistaken for a proper triplet; indeed, such a situation can be produced experimentally.[41] Finally, uracil occurs in almost all the triplets, although no naturally occurring RNA preparations have such a high uracil content. Unless there is a trivial explanation for this it might be that only a part of the cellular nucleic acids, high in uracil content, is used for coding of this sort, or alternatively that in the experiments described, only a part of the added synthetic RNA, lower than the average in uracil content, is functioning.

This tentative description of the code is supported by analyses of the amino acid composition of mutants of tobacco mosaic virus produced by infection of tobacco leaves with specifically altered tobacco-mosaic-virus RNA (as discussed below).[42] Additional confirmation can be found in the changes in amino acid sequence that occur in the various human hemoglobins.[43] It seems likely that this code will be universally applicable. Its formulation represents a major advance.[44]

TABLE 1. *The Postulated Triplet Code.*[39]

Amino Acid	Code*	Amino Acid	Code*
Alanine	UCG	Leucine	UUC (UUA, UUG)
Arginine	UCG	Lysine	UAA
Asparagine	UAA (UAC)	Methionine	UAG
Aspartic acid	UAG	Phenylalanine	UUU
Cysteine	UUG	Proline	UCC
Glutamic acid	UAG	Serine	UUC
Glutamine	UCG	Threonine	UAC (UCC)
Glycine	UGG	Tryptophan	UGG
Histidine	UAC	Tyrosine	UUA
Isoleucine	UUA	Valine	UUG

*U = uracil, C = cytosine, A = adenine, & G = guanine. Order of bases in each triplet not established; triplets in parentheses additional possibilities.

Mutations

The word "gene" now usually means a segment of the DNA that contains information about the structure of a certain protein. Large or small alterations (mutations) in this segment will lead to a change in the protein. The loss of a large piece of DNA results in no protein formation. Shorter deletions, or changes in perhaps even one base pair by, for example, erroneous base pairing between thymine and guanine, lead to a change in amino acid sequence and an enzyme that is partly or completely inactive, or altered in another respect. By precise genetic analysis changes in bases close together in DNA can be distinguished.[45] In addition to genes containing structural information there are genes that regulate the rate of formation of proteins (as discussed below). The mutation of such a gene can lead to the formation of an *increased* amount of protein, in contrast to the mutation of a structural gene, which would lead to a *decreased* amount of protein, or a less active one.

In addition to "spontaneous" mutants, a number of chemical and physical agents increase the frequency of mutations. A variety of mutant cells can be isolated through appropriate selective technics from bacterial cultures exposed to x-rays or ultraviolet light. Mutants that lack the ability to form certain essential metabolites can be obtained by the selective killing of wild-type cells; such mutants have been of great help in defining various metabolic pathways.[46] The extension of these technics to mammalian cells in culture has been limited so far to the selection of spontaneously occurring mutants, particularly those resistant to various drugs. As yet there have been no reports of mutagenesis in mammalian cell cultures.

Of the mutagenic agents now available, two relatively specific agents are illustrative. One, 5-bromouracil, is an analogue of thymine, the size of the bromine atom being close to that of the methyl group. Under certain conditions bacteriophages, bacteria and mammalian cells can be made to form DNA, in which much of the thymine is replaced with 5-bromouracil. At least with bacteriophages and bacteria this substitution causes an increased number of mutants in the survivors.

Recent data confirm the previous hypothesis that the substitution of 5-bromouracil for thymine leads to a base-pair change.[47] 5-Bromouracil is slightly more likely than thymine to hydrogen bond with guanine, and at the next duplication the erroneous guanine would pair with cytosine, converting the original adenine-thymine pair into guanine-cytosine. Other agents may act similarly, or may perhaps cause the substitution of a pyrimidine for a purine.[48]

Another relatively specific mutagenic agent is nitrous acid, which changes cytosine to uracil, and adenine to hypoxanthine, even when these bases are situated in a nucleic acid. It is possible to use a low concentration of nitrous acid, which can be expected to change but one base per nucleic acid molecule. This

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technic was first exploited with the tobacco mosaic virus, the nucleic acid of which is RNA (like many animal viruses). The viral RNA can be separated from its protein coat, treated with nitrous acid and then used to infect tobacco leaves either by itself or when recombined with viral protein.[49] Infection with slightly deaminated tobacco-mosaic-virus RNA leads to the production of mutant viruses. Correspondingly minor changes are present in the amino acid composition of the protein coats of such mutant viruses. Regarding the code described above, the changes in amino acid composition that are found would in the main be those expected, if in the viral RNA cytosine had been changed to uracil, or adenine to hypoxanthine.[42]

Transfer of Genetic Information

Information directing the formation of a new protein can be given to a bacterial cell by the introduction of new DNA. This fundamental phenomenon, called "transformation," was first characterized in 1944 by Avery, MacLeod and McCarty,[50] who showed that DNA-containing extracts could change the capsular antigens of pneumococci. Subsequently, it was found that various genetic information may be integrated into the DNA of recipient bacteria by this means, and it was essentially proved that DNA alone is sufficient.[51] It is known that a DNA molecule of certain minimum size is required, that genetic information is adequately contained in a single strand of DNA and that the recipient cells exhibit a cyclic competence to be transformed. The fate of the transforming DNA in the recipient bacterium has to some extent been elucidated. The other mechanisms of genetic transfer to be mentioned are more complex, if sometimes more efficient.

Multicellular organisms may not have retained the ability to transfer DNA intercellularly. However, the DNA of two tumor viruses is infectious, and Szybalski et al.[52] have described in brief what seems to be the successful transformation of sensitivity to the analogue 8-azaguanine in cultured mammalian cells. The drug-resistant cells lack the enzyme necessary to convert 8-azaguanine into 8-azaguanylic acid. Transformation could be detected when such resistant cells were exposed to DNA from the wild-type cells and acquired the enzyme. If substantiated, this discovery would initiate the genetic analysis of somatic mammalian cells.

In another type of DNA transfer the vector is a bacteriophage. Two kinds of bacteriophage infection occur[53]: that which is cytocidal and productive of new virus and that which is noncytocidal and nonproductive. In the former the genetic information in the bacteriophage DNA is immediately expressed in the bacterial cell cytoplasm, with the formation of messenger RNA and several enzymes that construct new viral DNA and protein. In the latter, which is called "lysogeny," the cell and its progeny are known to be infected because certain agents can cause them to produce virus, they are immune to superinfection and they may be altered in some observable way. Here, the DNA of the infecting virus is known by genetic analysis to be attached to a certain region of the cell DNA, different regions with different bacteriophages, and to be duplicated with it. Furthermore, the lysogenic bacteriophage may bring with it into the infected cell part of the DNA of the donor cell in which the bacteriophage was produced. By this phenomenon, which is called "transduction,"[54] a variety of genetic markers may be transferred from one cell to another, just as with transformation.

The fact that bacteriophage infection may be either of two types introduces the concept that DNA containing genetic information may be duplicated in the cytoplasm or in the chromosome. Genetic information with this choice has been called an "episome."[55] Another example of an episome is the fertility factor, discussed below. Furthermore, this factor, like a lysogenic bacteriophage, may bring with it into a recipient cell part of the genetic material of the donor cell, a process akin to transduction and called "sexduction." It becomes difficult to distinguish virus from normal cell constituent.

Whether lysogeny or transduction occurs in mammalian cells is not yet known. Since tumor viruses do not kill the cell that they infect the presence of a lysogenic infection is suggested. Pioneering experiments by Rubin and Temin[56] indicated that the Rous sarcoma virus caused a type of infection intermediate between cytocidal and lysogenic. More recent results with the mouse polyoma virus in cultured hamster cells have certain features consistent with lysogeny, but the analogy is incomplete in that the infected cell cannot be induced to form virus with chemical or physical agents and in that the infected cells may lose their immunity to challenge infection.[57] A promising newcomer to this field is the infection of cultured human cells with the SV40 virus.[58] Biochemical studies on the mechanism of oncogenesis in these systems are hampered by the low percentage of cells that as yet can be made neoplastic upon exposure to the virus.

DNA can also be transferred from one cell to another by mating. Although there are now some indications that mating occurs between somatic mammalian cells[59] the phenomenon has been studied extensively in bacteria.[55] Certain bacterial strains have been selected for frequency of mating. This ability seems to be related to the presence in these bacteria of a fertility factor capable of infecting other cells. Mating occurs via a cytoplasmic bridge between two bacterial cells, has a direction so that sexuality can be designated and probably consists of the transfer of DNA alone. Studies in which mating was inter-

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rupted have indicated that DNA is transferred in a certain order, which can be formulated most easily if the DNA of the donor bacterium is assumed to be circular. The fertility factor appears to determine the initial point of the transfer by attaching to the DNA and breaking the circle.

The intercellular transfer of genetic information can also be mediated by RNA. The best studied example is infection with the tobacco mosaic virus or its RNA; the RNA of several cytocidal animal viruses is also infectious. That viral RNA acts like messenger RNA is suggested by recent evidence that tobacco-mosaic-virus RNA stimulates the formation of a protein similar to virus protein when added to a cell-free protein-synthesizing system,[60] as well as recent studies with animal viruses.[61] Cellular as well as viral RNA can probably be transferred between cells. For example, it has been shown that chick fibroblasts exposed to RNA from *Esch. coli* form a protein that is immunologically similar to *Esch. coli* proteins.[62]

Control of Expression of Genetic Information

Jacob and Monod[18] have stated that "the fundamental problem of chemical physiology and embryology is to understand why tissue cells do not all express all the time all the potentialities inherent in their genome." Clearly, the flow of information from DNA to protein must be regulated in some way. Studies in recent years with bacteria indicate that many metabolic processes are controlled by direct feedback mechanisms.[63] For example, the final product of several sequential enzymatic reactions may inhibit the activity of the initial enzyme in the series.

Besides feedback inhibition, it has been known for some time that many enzymes vary in *amount.* Cells exposed to certain substrates produce enzymes to catabolize these substrates. Conversely, if cells are supplied with certain products such as amino acids, they repress the formation of the enzymes usually responsible for forming such products. Recent results indicate that induction and repression of enzyme formation occur in mammalian cells as well as bacteria.[64,65]

How is the rate of enzyme formation controlled? From their own work and that of others, Jacob and Monod[18] have formulated the following hypothesis: the control mechanism operates at the genetic level by regulating the activity of genes with structural information; in addition to "structural" genes, "regulator" and very probably "operator" genes occur; and for all inducible or repressible enzymes, perhaps for all proteins, regulation consists of various degrees of inhibition of formation (Fig. 3). It is suggested that an operator gene controls the initiation of formation of messenger RNA on one or more structural genes and that a regulator gene forms a cytoplasmic "repressor" molecule (? another type of RNA), which

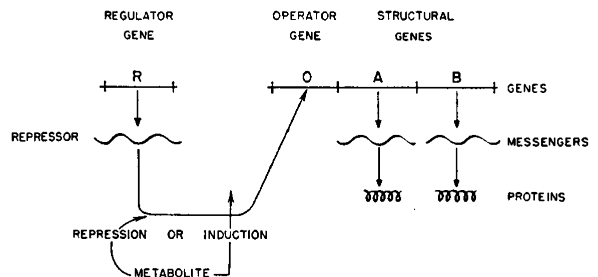

FIGURE 3. *Hypothesis of Jacob and Monod[18] for the Regulation of Protein Synthesis.*

tends to combine with and block the operator gene. Substrates that induce enzyme formation would somehow inactivate this repressor, and products that repress enzyme formation would activate it — induction equals "derepression."

Several lines of evidence support the concept of a regulator gene, as an entity distinct from the structural gene. For example, it is possible to isolate bacterial mutants that in the absence of inducer produce more of an enzyme than can be found when the wild-type bacteria are maximally induced. Likewise, nonrepressible mutants of repressible bacteria occur. By genetic analysis the chromosomal location of these mutations can be shown to be separate from the location of the structural genes. Studies with artificial heterozygotes indicate that the inducible and repressible states are dominant. Therefore, it seems likely that it is in the inducible or repressible bacteria that the regulator gene is active.

That regulator-gene activity consists of the formation of a cytoplasmic repressor is suggested especially by certain mating experiments. It can be shown that when male bacteria, which lack the enzyme beta galactosidase but can be induced to form it, are mated to female bacteria unable to form beta galactosidase, enzyme formation starts immediately in the zygote *without the presence of inducer.* This suggests a difference in the cytoplasm of the two bacteria, since only DNA is transferred. Enzyme synthesis ceases after about an hour, but can be made to reappear by the addition of inducer at that time. The most obvious interpretation of these experiments is that in the cytoplasm of the donor male cell, there is a repressor, which is not present initially in the female cell. The newly acquired DNA causes the formation of both enzyme and repressor, but it takes an hour to build up an effective concentration of the latter. Then the situation is similar to the one that existed in the donor cell: for further enzyme synthesis to occur, inducer must be added to antagonize the repressor.

In such an experiment it is possible to use donor DNA containing the DNA of a lysogenic bacteriophage. Upon introduction into a nonlysogenic female bacterium bacteriophage synthesis begins immediately. These results and other aspects of lysogeny, such as immunity to superinfection, suggest that the DNA

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of the lysogenic bacteriophage produces in the host cell a repressor molecule, which prevents this DNA from directing the formation of the proteins concerned with new bacteriophage production. Induction by chemical or physical agents might somehow inhibit the formation of the repressor and allow the formation of these proteins. The decision whether or not infection with a bacteriophage will be productive or lysogenic would depend upon whether messenger RNA or repressor was formed first. It is interesting in this regard that several of the tumor viruses may be cytocidal, rather than oncogenic, in certain situations.

Discussion

Some of the concepts outlined in this review are illustrated by the way in which a cell might control the synthesis of its DNA. The growth of a mammalian cell from one mitosis to the next is characterized by a changing pattern of synthetic processes.[66,67] The formation of DNA is a discrete event, occurring late in interphase and presumably interrupting the transfer of information from DNA to RNA. What begins the duplication of DNA and ends it so precisely? Are the enzymes that form the deoxyribonucleoside triphosphates derepressed to bring each triphosphate to an optimal concentration? Does the DNA polymerase enzyme increase in amount, or move closer to the DNA? What initiates the separation of DNA into two strands? When the two new double helixes are completed, why are they not subject to separation and duplication? At that point does the RNA polymerase displace the DNA polymerase from the DNA? In duplicating itself the dual functions of DNA become tangled indeed.

These concepts have encouraged a fresh approach to the problems of differentiation and neoplasia. The former may involve changes in the rate of formation of various enzymes, as suggested by studies with regenerating liver,[68] and the latter, presumably a mutation, changes in the concentration of key synthetic enzymes[69] or perhaps the alteration of certain cell-wall proteins.[70] On the other hand, either phenomenon may result from the intercellular transfer of genetic information.[71,72]

The past ten years have produced a remarkable development in knowledge of genetic information and the mechanism of its expression. The major phenomena seem established although various details are as yet unclear. The application of this knowledge to the many related problems in biology and medicine lies ahead. Here again, "What's past is prologue."

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MEDICAL INTELLIGENCE

CANDIDA MENINGITIS SUCCESSFULLY TREATED WITH AMPHOTERICIN B*

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THE following is the twenty-second case of candidiasis of the central nervous system reported since 1933[1-21] and the second to have been treated successfully with amphotericin B.

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Case Report

A 1-month-old male infant weighing 2.9 kg. (6 pounds, 7 ounces) was admitted to the hospital on November 30, 1960, with candida enteritis of 3 weeks' duration.[22] This condition subsided within 3 days of oral administration of nystatin, 100,000 units every 8 hours. Isolation of a coagulase-positive *Staphylococcus aureus* from the blood necessitated intravenous infusion of chloramphenicol, 150 mg. per day, and penicillin, 2,000,000 units daily, for 7 days.

A subsequent blood culture yielded *Candida albicans*, coinciding clinically with nuchal rigidity, twitching of the facial muscles, hypertonia of the limbs and temperatures of 103 to 104°F. There were no other localizing neurologic signs. A spinal tap revealed a normal cerebrospinal fluid as far as chemical constituents, cell counts and culture were concerned. The 2d specimen, obtained on the 49th hospital day, was clear, with 48 per cent polymorphonuclear cells and 52 per cent lymphocytes, normal chemical constituents and negative cultures for bacteria and fungi.

Stool cultures remained positive for *C. albicans*. Recurrent episodes of oral and cutaneous candidiasis were treated locally with nystatin suspension (4 100,000 units) swabbed on the oral mucous membranes every 6 hours, and nystatin ointment, applied 3 times a day, respectively.

Persistent flexion of the thighs at the hip joints, with obvious pain on extension, developed. These symptoms were ascribed to osteomyelitis as revealed by x-ray study of both humeral and the right femoral heads. After 6 weeks of intravenous therapy with methicillin, 600 mg. daily, the temperature subsided temporarily, the motility of the thighs and hips improved, and x-ray examination showed considerable improvement of the osteomyelitis.

During the 3d and 4th hospital months the temperature rose again despite continued methicillin therapy. Successive cerebrospinal-fluid specimens now showed a marked rise in polymorphonuclear cells and in protein, decreased glucose levels, and consistently yielded *C. albicans* in cultures.

Amphotericin B, 37.5 mg., was infused intravenously between the 135th and 205th hospital days and had to be discontinued because of thrombophlebitis. The abnormalities and cultural findings of the cerebrospinal fluid persisted although the patient was subjectively well and showed no neurologic symptoms (Fig. 1).

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