Nucleic Acid Synthesis in the Neoplastic Cell and Impact of Nuclear Changes on the Biochemistry of Tumor Tissue: A Review

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INTRODUCTION

The spectacular progress in nucleic acid chemistry, bacterial genetics, and viral biology has stimulated renewed efforts at an integration of the two major theories of carcinogenesis—the somatic mutation theory, and the viral theory. Four converging lines of investigation have provided the basis for such an integration: (a) The evidence that nucleic acids constitute the probable repositories of genetic information (44); (b) the discovery that the replication of an entire virus can be initiated following cell infection with the appropriate viral nucleic acid alone (53, 83, 104); (c) the finding that deoxyribonucleic acid (DNA) is capable of effecting genetic transformations in microorganisms (206, 232); and (d) the analyses of lysogeny and transduction (1). Once the results of these studies were appreciated, it was inevitable that their implications for oncology should be pointed out (6, 7, 242).

The integrative theories of cancer are, of necessity, preliminary in nature. This follows from the fact that many of the central issues of biology are unresolved and the fact that the areas of nucleic acid chemistry, microbiology, and genetics are still in a state of rapid development. Evidence in animal cells for a “lysogenic-like” integration of tumor-inducing viruses is as yet only circumstantial (189, 235). The chemistry of nucleic acid replication is by no means settled; and the status of ribonucleic acid (RNA) viruses in the over-all scheme remains in doubt.

Nevertheless, the present paper starts with the assumptions that the viral and somatic mutation theories of cancer are indeed amenable to synthesis into a larger whole, and that both viral and somatic mutation carcinogenesis are expressed through common mechanisms. The following quotation from Luria is noteworthy, “The examples discussed (in reference 146) illustrate known instances of mutational changes from genetic elements that behave as nonviral determinants of heredity. These mutational transitions and the similarity of gene action exerted by viruses in destructive and in persistent types of infection indicate that gene control and virus control over cellular functions are two aspects of the same genetic mechanism. This concept removes any a priori incompatibility between a viral and a genetic theory of cancer etiology. It reduces the interpretation of virus-induced cancer to that of the control of cellular development and differentiation by genetic elements capable, either intrinsically, or by association with other specialized genetic elements, of assuming an infectively transmissible form.”

This essay constitutes a summary of current biochemical thinking on the problems of gene action and the control of metabolism. The plan is as follows: First, the hypothesis that neoplasia, like mutation, results from disturbances in the replication and function of nucleic acids will be discussed. The action of carcinogenic and mutagenic agents at the molecular level will be indicated. Secondly, attention will be directed to the structure, composition, and conformation of the nucleic acids. This will be followed by an analysis of the experimental evidence for the proposed Watson-Crick models of DNA replication. Suggested biochemical mechanisms for errors in DNA replication will then be considered. Such errors represent the chemical basis for gene mutation.

The question will then be raised whether bio-
chemical differences between the DNA of normal and tumor tissues can be experimentally demonstrated. Data on the molecular base composition, the range of compositional heterogeneity, the chromatographic heterogeneity, and the total cellular DNA content of tissues will be reviewed. It will be shown that estimations of the DNA content support the concept that a remodeling of the genetic elements has taken place in neoplasia. The consequences of the genetic reorganization will then be considered. Three aspects of gene action and metabolic control will be distinguished: (a) control at the gene level; (b) control at the level of the enzyme-forming system; and (c) at the enzyme level itself. At the gene level, this will entail a discussion of cell architecture, the effect of gene mutation on protein and enzyme structure and composition, the effects of polyploidy, heteroploidy, and multiple gene dosage, and the consequences of the acquisition by a cell of genetic elements from without. The discussion of control at the level of the enzyme-forming system will involve the phenomena of enzyme repression and induction, a consideration of the sights of synthesis of RNA, and the properties of the ribosomes. An approximate estimate will be presented of the number of enzyme-forming templates in bacterial cells and lymphoma cells. Finally, in relation to control at the enzyme level, we will consider the suppressor gene phenomenon, negative feedback mechanisms, the dissociation of macromolecules which result in apparent enzyme activation, and the more familiar processes of the control of enzyme kinetics.

**Disturbances of Nucleic Acid Metabolism and Carcinogenesis**

As pointed out by Auerbach (146, 147) and Barratt and Tatum (14), many carcinogenic agents are mutagenic in Drosophila and in microorganisms. Among these are x-rays, ultraviolet light, nitrogen mustards, urethan, dibenzanthracene, methylaminoanthrene, benzpyrene, m'-methyl-p-dimethylaminoazobenzene, aminoaazotoluene, and tert-butyl peroxide. Some carcinogens which do not induce gene mutations do, however, increase the incidence of chromosomal breaks and translocations. Among the carcinogenic or mutagenic agents which induce chromosomal translocations and fragmentations are nitrogen and sulfur mustards, radiation, diepoxides, urethan, aminoacridines, benzpyrene, methylaminoanthrene (28), and p-dimethylaminoazobenzene (212). In addition, Lwoff has emphasized (148) that a number of the carcinogen-mutagens induce prophage development in lysogenic bacteria.

Although the picture is far too complicated to be resolved by any facile and all-embracing generalization, it is apparent that disturbances of nucleic acid metabolism may in several instances represent the common basis for action. The nature of the nucleic acid disturbances need not be the same, and the initial effects may vary, but the end-results would be modifications of genetic structure and function. The following salient points may be cited:

1. Stanfield Rogers has shown (187, 188) that disturbances of nucleic acid metabolism are critically involved in carcinogenesis by urethan and nitrogen mustards.

2. BieseI has demonstrated (27, 28) that azaserine, 6-diazo-5-oxo-L-norleucine (DON), mercaptopyrimidine riboside and thioguanine riboside increase the frequency of mitotic abnormalities in fibroblast and sarcoma cell cultures. These substances are antimetabolites of nucleic acid precursors. Azaserine is also a potent bacterial mutagen.

3. Mitotic abnormalities and chromosomal aberrations are frequently encountered in primary tumors as well as in tumors of long standing (81, 97, 100, 140, 215, 230). The chromosomal abnormalities may include aneuploidy, heteroploidy, and quasidiploidy, alterations in chromosomal morphology including drastic chromosomal rearrangements, and the presence of marker chromosomes. In instances where chromosomal abnormalities have not been detected, there is the suspicion that they may, nevertheless, exist and that their elucidation may be a function of the resolving power of existing cytological tools. Moreover, the absence of gross chromosomal changes need not rule out more subtle alterations of the genetic elements.

4. A number of viruses are replicated in the cell nucleus, where they induce manifestations of nucleolar enlargement and hyperactivity as shown by increases in the RNA mass of the nucleolus, in some instances at the point of attachment of the nucleolus to the chromosomes (70, 71). Viral synthesis in the nuclei of cells has been demonstrated by electron microscopy (11, 67, 159, 160, 217, 218), fluorescent antibody techniques (101), biochemical (163), cytological, and autoradiographic methods (60, 70, 71, 145). Viruses which proliferate in the nucleus include: polyoma virus (11, 67, 101), the Shope papilloma virus (159, 218), the virus which induces adenocarcinomas in amphibian renal tissues (70, 71), RPL-12 lymphomatosis virus (60), herpes simplex virus (163, 217), and the adenoviruses (160).
Disturbances of nucleic acid metabolism could take one of several forms. For example:

a) Radiation inhibits the synthesis of nucleic acids in microorganisms and a variety of animal cells (18, 37, 119, 148). The effects may be ascribed to inhibition of key enzymes or, possibly, to damage to templates. Irradiation in vitro induces partial degradation of DNA which may be detected by viscosity measurements (2, 3, 195) or by chromatographic procedures (20). Irradiation of rats produces an increase in that part of the DNA which is eluted from anion exchangers by 2 M NaCl/0.1 M NH₃ at the expense of the less easily eluted components (167). Ultraviolet light causes a 1000-fold inactivation of H. influenzae-transforming factor (252).

b) Nitrogen and sulfur mustards inhibit DNA synthesis in growing cultures of E. coli, in tissue culture cells, animal cells in vitro, and animal tissues in vivo (68, 96). The biological activity of transforming principle is lost upon treatment with nitrogen mustard even in concentrations which do not affect the viscosity (252). In vitro, nitrogen mustards esterify the phosphate groups of DNA and RNA (207). Polyfunctional alkylating agents can react with two different nucleophilic centers to form a bridge between them. In dilute nucleic acid solutions, intramolecular cross-linking can take place with the formation of a more compact molecular configuration (2, 3, 207). This is manifested by a decrease in the viscosity and the radius of gyration of the molecules. Immediately after reaction with nitrogen mustards, the molecular weight of DNA is not reduced, but it falls on standing at 37°C, because the triphosphate esters produced by the alkylating agents are unstable and hydrolyze. DNA alterations are also manifest from the study of the DNA chromatographic profiles on ECTEOLA-cellulose exchangers (20). At higher DNA concentrations (sperm), bifunctional agents cross-link different DNA molecules together via their phosphate groups. It is improbable that the adenine and cytosine amino groups of DNA are the primary points of attachment by the nitrogen mustards, since these groups are hydrogen-bonded in the native structure. Although both nitrogen mustards and radiation reduce the viscosity of DNA, the mechanisms may be different. The chemical changes due to x-rays are brought about by the highly reactive free radicals (such as OH·) which are formed on irradiating water. Whereas radiation lowers the viscosity by degrading the polymers into smaller molecules, the mustards achieve the same effect by internally cross-linking the molecules so that they occupy smaller volumes in solution (2, 3).

c) Although amino acridine derivatives have not been shown to possess carcinogenic activity, these substances are strongly mutagenic to Dro sophila (6), bacteriophage (1, 40), bacteria (241), yeast (76), and polio virus (69). The amino acridines form complex salts with the phosphate groups of nucleic acids (16, 39). When bacteria are infected with T₂ bacteriophage in the presence of proflavine, synthesis of protein and DNA occurs, and the bacteria lyse as usual but do not liberate infective particles (1). The bacterial lysates obtained in the presence of proflavine contain the same “doughnuts” (empty phage heads) that have been seen in premature lysates not containing proflavine. The phage heads contain sulfur and phage-specific complement-fixing antigens but no DNA. Free DNA containing hydroxymethyl cytosine is also found, showing that phage-specific DNA has been formed. Phage production seems to be arrested at a late stage that may call for the assembly of these several components. Acridine orange is a potent photosensitizer. The effects of the dye may in part be mediated through its photosensitizing action (107). When animal or bacterial cells are grown in the presence of acridine orange in the dark, there is no appreciable effect on rate of multiplication, replication of DNA, or protein synthesis. When the cultures are not shielded from light, these processes are inhibited.

Unlike the aminoacridines, various benzacridine derivatives (i.e., methyl-1,2 benzacridine) are strongly carcinogenic (10, 136). Benzacridine salts, like those of acridine itself, have a strong photodynamic effect on Paramecia. Lacassagne et al. (136) have emphasized that, in a series of methyl benzacridines, the probability of finding a carcinogenic compound increases with the increase in charge of the K region calculated by theoretical methods.

d) Although bromodeoxyuridine has not as yet been shown to be carcinogenic, this substance induces marked chromosomal abnormalities in tissue culture cells. Bromodeoxyuridine is mutagenic in bacteria (253) and bacteriophage (40, 41, 143). The mutagenic action of 5-bromouracil is highly specific. Comparison of the distribution of R₄ mutations induced by proflavin and by 5-bromouracil shows that these two mutagens act on completely different groups of sites in the genetic structure (40) and also at points differing from those at which

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1 D. Billen and T. C. Hsu, personal communication.
spontaneous mutation most frequently occurs. Bromodeoxyuridine is incorporated in place of thymine into the DNA of bacteria, bacteriophage, and animal cells (41, 92, 143, 253). It has been shown at the enzyme level that DNA polymerase can incorporate the deoxyribonucleotide triphosphates of several base analogs into DNA. Uracil and bromouracil specifically replaced thymine; 5-methyl and 5-bromocytosine replaced cytosine; and hypoxanthine replaced guanine (24).

The mutagenic effect of bromodeoxyuridine on phage is found even when the thymine analog is taken up by infected bacteria in the presence of chloramphenicol, i.e., when protein synthesis is strongly inhibited. This result implies that DNA formed in the presence of chloramphenicol is genetically active and also that protein synthesis is not essential in initiating the series of events leading to mutation by bromodeoxyuridine. This supports the conclusion that the mutagenic action of bromodeoxyuridine results from its incorporation into DNA—the eventual creation of a difference in natural base sequence being the actual mutational event.

Other pyrimidine and purine analogs may be incorporated into DNA and RNA. Iododeoxyuridine may replace thymine in the DNA of human leukemic leukocytes following the in vivo administration of the analog. Iododeoxyuridine is also incorporated into DNA of tissue culture cells (73). Fluorouracil, an inhibitor of RNA synthesis and of the methylation of deoxyuridylic acid to thy- midylic acid, is itself incorporated in place of uracil into the RNA of bacteria (110), animal cells (47), and tobacco mosaic virus (89, 208). That the presence of fluorouracil in the RNA affects nucleic acid function is suggested by the fact that fluorouracil almost entirely blocks the induction of β-galactosidase in E. coli. Moreover, the fluoropyrimidine prevents any further rise of the β-galactosidase activity in cells that have previously been treated with the inducer or in a strain of E. coli in which this enzyme is constitutive. Thiouracil and 8-azaguanine are other analogs which are incorporated into the RNA of tobacco mosaic virus (89).

The Structure and Conformation of DNA and RNA

For a proper appreciation of nucleic acid function, a few words are required on the structure and conformation of DNA and RNA. It is now generally accepted that the Watson-Crick model (237) adequately describes the properties of most DNA molecules. On the basis of x-ray diffraction studies of DNA from a variety of sources, Watson and Crick constructed DNA models and made the now classic proposal that DNA consists of two helical polynucleotide chains of opposite polarity which are twined round one another. The two chains are held together by hydrogen bonds between the bases, each base being joined to a companion base on the other chain. The pairing of bases is specific, adenine going with thymine and guanine with cytosine. The phosphate groups are accessible to hydrogen or hydroxyl ions and to dyes and are therefore on the outside, whereas the bases occur opposite one another on the inside. Prominent 3.36Å spacings along the fiber axis were interpreted as due to a succession of flat nucleotides standing out perpendicular to the fiber axis to form a relatively rigid structure and consistent with the high density of DNA.

The concept of DNA as a stiff-coiled macromolecule is also based upon physico-chemical data which suggest particles having a molecular weight of $10^6$ to $10^7$, an axial ratio of 300:1, a diameter of about 500 μm, and a thickness of about 2.5 μm. This view gains additional support from direct observations with the electron microscope, which show the molecules to be threadlike and stiff, with a thickness of 1.5–2.0 μm (for references, see 120).

Physico-chemical evidence favoring the Watson-Crick model of DNA includes: (a) titration curves which suggest that the bases are hydrogen-bonded; (b) the shape and size of the molecule in solution obtained from a combination of light scattering, viscosity, and sedimentation measurements (175), which show that DNA in solution is highly extended but not completely straight, and that its diameter is compatible with the double helix model; (c) kinetic studies on the degradation of DNA by x-rays, acid, or enzymes, which are consistent with there being two strands in DNA so that the molecules do not come apart until there are breaks in both backbones opposite one another; (d) chemical analyses of the molar ratios of purine and pyrimidine bases (245); and (e) experiments on the effect of heat on the physico-chemical properties, ultraviolet absorption spectra, chromatography, and biological activity of DNA. The Watson-Crick model has gained additional support from the recent biological and physico-chemical studies by Marmur and co-workers (65, 133) on the separation and specific recombination of the strands of the DNA duplex.

There is one well authenticated instance of a DNA with an exceptional structure. This is the DNA of bacteriophage φX174, which seems to be single-stranded (200). The DNA of phage S13 may also be single-stranded, and it is conceivable that other examples will be encountered (225).
The RNA of animal cells, bacteria, and TMV-virus also appear to be single-stranded macromolecules containing helical regions and intramolecular hydrogen bonding (125). Evidence for the single-stranded coiled structure of RNA has been derived from six experimental approaches: (a) measurement of the changes in optical density with temperature, formaldehyde, or cation concentration; (b) experiments on the optical rotation of RNA under various conditions; (c) physicochemical studies of the sedimentation rate, viscosity, or light scattering properties of RNA; (d) investigations of the chromatography of RNA on anion exchangers; (e) x-ray diffraction experiments; and (f) studies of the rate of RNA degradation and the inactivation of biological activity by heat or enzymes (for references and details, see [125]). In addition, RNA molecules from a number of sources do not show in their purine and pyrimidine molar base composition an equivalence of adenine with uracil and of cytosine with guanine. This is in contradistinction to DNA.

Sedimentation studies in the analytical ultracentrifuge of bacterial or animal tissue RNA reveal the presence of two components having sedimentation constants of about 30S and 18S. The sedimentation constants of RNA solutions decrease as the ionic strength of the medium is decreased, whereas the viscosities increase. The viscosity at elevated temperatures is greater than that at room temperature. A reversible hyperchromicity is manifested by RNA solutions when the temperature of the solutions is raised to 95°C. The optical density-temperature curves for RNA differ markedly from those of native DNA. In the case of RNA, the optical density of the solutions begins to increase at a temperature of about 30°–40°C, and a gradual increase of about 30 per cent is observed as the temperature is raised to 95°C. The curves may be shifted to higher temperatures by increasing the ionic strength of the solution. Unlike native DNA, RNA reacts rapidly with formaldehyde; the rate of the reaction is temperature-dependent. These observations are all consistent with the hypothetical structure of RNA as a single-stranded, coiled polynucleotide with partial intramolecular hydrogen bonding. The hyperchromicity with temperature, and dependence of viscosity, sedimentation, and reaction with formaldehyde on temperature are all indicative of alternative structures of the molecules in solution so that the molecules are more extended and less hydrogen-bonded at low ionic strengths and higher temperatures (94, 125, 144, 227). The fact that RNA and denatured DNA both manifest hyperchromicity and a shift in the maximum of the absorption curve after reaction with formaldehyde, whereas native DNA does not, suggests that the amino groups of adenine and cytosine participate in forming the hydrogen-bonded structure (82).

The two RNA components of bacteria, tumor cells, and animal tissues have molecular weights of the order of magnitude of 1 and 2 × 10^6, respectively (125, 144, 227). High molecular weight RNA has also been isolated from liver microsomes (50, 94) and calf thymus nuclei (49). The molecular weight of tobacco mosaic virus RNA and of other RNA viruses seems to be about 1.7 × 10^6 (29, 49).

DNA SYNTHESIS

Mechanisms.—A plausible mechanism for DNA replication has been suggested by Watson and Crick (237). According to this proposal, the twin-stranded DNA molecule partially unwinds, and each base attracts a complimentary free nucleotide already available for polymerization within the cell. These free nucleotides, whose phosphate groups probably already possess the free energy necessary for polymerization, would then link up with one another after being held in place by the parental template chain to form a new polynucleotide molecule of the required nucleotide sequence. Thus, each DNA strand serves as a template for the synthesis of a complementary strand. This hypothesis of DNA replication falls in the class of semiconservative mechanisms of replication in contradistinction to conservative or dispersive mechanisms (61). Conservative mechanisms are those which do not destroy the integrity of the entire parental DNA duplex in the course of the replication process so that, among the daughter duplexes produced by one or more replications, there is one which is entirely parental and the rest entirely new. This could be true, for instance, if DNA were replicated indirectly through the intermediary of another substance, e.g., protein or RNA, which was first synthesized on the surface of the intact parental duplex and then served as a template for the synthesis of the daughter DNA duplexes. Semi-conservative mechanisms are those which conserve the atomic identity of single chains of the parental DNA duplex, although effecting a permanent separation of the two chains from each other in the course of replication. Dispersive mechanisms are those which do not conserve the atomic integrity of the chains of the parental duplex but result in the dispersion of its atoms among the replica duplexes. A scheme which involves numerous breaks in the original chains falls into this class.
The evidence for the Watson-Crick semi-conservative mechanism of DNA replication is impressive but by no means conclusive. Four lines of experimental evidence are relevant: (a) equilibrium sedimentation studies in a CsCl gradient of labeled progeny DNA (157, 198, 219); (b) radioautographic studies of chromosome replication (224); (c) the enzymatic replication of DNA (138, 139); and (d) various bacteriophage studies of progeny formation (141, 209, 210).

One method of testing the Watson-Crick hypothesis of DNA replication is the "transfer experiment." In such an experiment, the distribution of labeled parental DNA to progeny molecules is followed. The Watson-Crick hypothesis makes some striking predictions concerning its outcome: (a) After just one generation in the non-labeling medium, each DNA molecule will be only half labeled; (b) at any generation after the first, there will be found only two classes of molecules, half labeled and completely unlabeled. For example, at the second generation, there will be equal numbers of half labeled and completely unlabeled molecules.

In their transfer experiments, Meselson and Stahl permitted N15-labeled E. coli to multiply in N14 medium. At intervals during the following four generations, samples of the DNA were examined with respect to the distribution of N15 label. This was accomplished by determining the density distribution of the DNA by the method of equilibrium density-gradient centrifugation. It was found that, when the populations of cells and hence of DNA molecules had just doubled, all the DNA molecules in the culture were exactly half-labeled. After just two doublings of the population, half-labeled and completely unlabeled molecules were present in equal numbers. Indeed, in all samples taken after the first generation, the fraction of DNA molecules which were half-labeled was 2^-k. These results demonstrate that the DNA molecules of E. coli are composed of two sub-units and that, upon duplication, each daughter molecule receives one parental sub-unit. The sub-units remain intact through many successive duplications.

The Meselson-Stahl experiments have been confirmed by Sueoka (219) who studied mitotic replication of DNA in the unicellular green alga, Chlamydomonas reinhardi, and by Simon (198), who carried out transfer experiments with HeLa cells in tissue culture. In the latter instance, the DNA was labeled with bromouracil. Transfer experiments of parental DNA to the progeny of bacteriophage φX-174, however, yielded a different result. When Kozinski and Szybalski (135) grew bacteria on a bromouracil-free medium and infected the bacteria with bromouracil-containing ("heavy") phage, no "heavy" phage was detected by CsCl centrifugation, even among the early phage progeny released by prematurely opened cells. Similarly, no UV-resistant bromouracil-free phage could be found among early phage progeny released by bromouracil-labeled bacteria infected with bromouracil-free phage. The results were interpreted as indicative of a dispersive mode of DNA replication in phage φX-174. It is possible that the mechanism of DNA replication in phage φX-174 differs from that of bacteria, algae, and animal cells. However, the phage experiments are complicated by the fact that the phage DNA undergoes many more replications prior to study than does the DNA of the higher organisms. Since the results are essentially negative in character, that is, the "heavy" DNA label of the parental phage was not recovered, the crucial question of the sensitivity of the method must be raised.

There is, however, another reservation to the interpretation of the Meselson-Stahl experiment. This is the question of whether the sub-units of DNA represent the complementary DNA strands of the Watson-Crick model or monomers arising from aggregates of DNA (157). On the basis of light scattering measurements of the molecular weight of DNA and of the kinetics of deoxyribonuclease degradation of heated E. coli DNA preparations, Cavaleri and co-workers have suggested that the unit DNA molecule of E. coli is actually a dimer composed of two double helices, laterally bonded together (45). Thus, each double helix would be conserved intact during cell division and the bonds holding the dimer together would presumably be ruptured during some part of the replication cycle. On the basis of this interpretation, the replication of DNA would be conservative rather than semi-conservative.

An unambiguous answer to this problem cannot be derived from the other experiments previously cited. Taylor and co-workers (224) labeled Vicia root tip chromosomes by immersing this actively growing region of the plant for a short time in liquid medium containing tritium-labeled thymidine. They then observed the distribution of label among chromosomes autoradiographically after one or two duplications in the absence of labeled thymidine. After one duplication, the two daughter chromosomes were seen to be labeled equally. Following the second duplication, two of the four chromosomes were usually labeled to the same extent as the first generation chromosomes; the other two were generally completely unlabeled. Objections to the use of colchicine to induce mitotic
arrest in these experiments (137) are discussed by Woods and Schairer (243). One must conclude from the results that a chromosome is composed of two sub-units which segregate, one into each daughter chromosome, and that each sub-unit is transmitted intact throughout successive generations. Here again, it cannot be stated with certainty that the sub-units represent DNA duplexes.

From the "star" experiments of Levinthal and Thomas (61, 141), there is evidence that a large piece of the transferred parental DNA of T-even bacteriophage is conserved, but the remainder of the transferred parental label is more widely distributed. In other experiments, Stent and co-workers (209, 210) estimated the distribution of the transferred DNA atoms by means of the lethal effects on the progeny population of the decay of bacteriophage. In the process of transfer from the transferred DNA atoms by means of the lethal effects on the progeny population of the decay of bacteriophage, it cannot be stated with certainty that the sub-units represent DNA duplexes.

The enzyme is absent or low in nondividing tissues such as normal rat liver, brain, or skeletal muscle, but appears at the same time (10 hours after the operation) as DNA synthesis in vivo in regenerating rat liver (34). When partially hepatectomized rats were irradiated 6–19.5 hours after the operation with x-rays, the radiation produced essentially complete inhibition of the increase in DNA polymerase (32) that would be expected to occur between 6 and 24 hours after partial hepatectomy.

A primer DNA is required for enzymatic activity. The *E. coli* polymerase is able to utilize various bacterial DNA's or calf thymus DNA as primers. Interestingly enough, the enzymatically synthesized DNA manifested the same molar base composition as the primer which was employed (138). As in the case of native DNA, the product of enzymatic synthesis showed an equivalence of adenine to thymine and guanine to cytosine. The single-stranded 5X-174 DNA also served as primer. In this instance, a double-stranded product was formed. Heat-denatured DNA is a more effective primer than native DNA (31, 138). From these findings, it is clear that the equivalence of adenine to thymine and guanine to cytosine is an inherent feature of DNA synthesis by the polymerase enzyme. Furthermore, the data suggest that the DNA added to the reaction serves as a template for the enzymatic replication of DNA.

If primer DNA is actually single-stranded DNA and if the synthetic reaction produces double-stranded molecules, then the idealized reaction should cease when the primer DNA has been doubled in amount. DNA increases of greater than twofold would be possible only with enzyme systems containing nucleases capable of converting native DNA to denatured DNA. Actually, net increases in DNA in amounts at least 10 times as great as the primer added have been observed (138). The explanation for this phenomenon remains to be elucidated.

To date, it has not been shown that enzymatically synthesized DNA is biologically active.

**Errors in DNA replication.**—It is a corollary of the Watson-Crick hypothesis concerning the structure and function of DNA that a change in the DNA sequence of one or a few nucleotides will be mutagenic (21, 40, 237). Mechanisms for spontaneous mutation and for experimentally induced mutations have been suggested on the basis of this concept. Watson and Crick pointed out (237) that the specificity in DNA structure (adenine pairing with thymine and guanine with cytosine) results from the assumption that each of the bases possesses one tautomeric form which is very much more stable than any of the other possibilities. The fact that a compound is tautomeric, however, means that the hydrogen atoms can occasionally change their locations. Thus, a spontaneous mutation might be owing to a base occurring very occasionally in one of the less likely tautomeric forms, at the moment when the complementary chain is being formed. For example, whereas adenine will normally pair with thymine, if there is a tautomeric shift of one of its hydrogen atoms, it can pair with cytosine. The next time pairing occurs, the adenine (having resumed its more usual tautomeric form) will pair with thymine, but the
cytosine will pair with guanine, and so a change in the sequence of bases will have occurred.

Nitrous acid is a mutagenic agent for tobacco mosaic virus, φX-174 phage, T4 phage (326), and poliovirus (30). Nucleic acids are deaminated by nitrous acid so that adenine is converted to hypoxanthine, guanine to xanthine, and cytosine to uracil. The production of mutants by nitrous acid is a one-hit process, the deamination of a single base presumably being sufficient to produce a mutant. In the case of RNA, the direct formation of uracil from cytosine can, obviously, account for mutations. However, the deaminations of adenine and guanine in RNA are not so obviously mutagenic, since one or more additional steps would be required in the host cell to replace the abnormal bases by normal ones in the new RNA chain. The abnormal bases could provoke a pairing error leading to a new sequence of nucleotides in the progeny RNA. In the case of DNA, none of the deamination products would be normal bases, so that all the nitrous acid-induced mutations might result from some sort of pairing error provoked during duplication by the presence of an abnormal base. The deamination products are essentially base analogs comparable to bromouracil and 2-aminopurine (41, 84, 143).

There is a clear-cut distinction between the mutagenic action of nitrous acid on φX-174 and T4 phage. Mutants of φX-174 arise in pure clones, whereas most T4 mutants arise in mixed clones, suggesting that there is only one copy of genetic information in φX-174 and at least two copies in T4. The alteration of one of the T4 copies yields a type of heterozygote. The number of copies of genetic information can be directly related to the apparent number of strands in the DNA molecule. It is inferred that heterozygotes induced by nitrous acid can consist of double-stranded DNA with singular regions in which the base pairs are not the normal complementary ones. These results are consistent with the picture that the DNA is in the Watson-Crick double-stranded configuration, both complementary strands carrying the same information. This conclusion is also strongly supported by the experiments of Marmur and Doty (168) in which hybrid DNA duplexes have been formed by the exchange of the dissociated double strands of wild type and homologous mutant DNA.

Mutants of T4 phage induced by bromodeoxyuridine and 2-aminopurine in the forward reaction can be induced to revert (84). In contrast, proflavine-induced and most spontaneous mutants cannot be induced to revert by bromouracil and 2-aminopurine, although they revert spontaneously. The difference between reverting mutants inducible and noninducible by the above two base analogs is so drastic that it seems to reveal two kinds of fundamentally different mutagenic effects: (a) a mutagenic effect by which the analog induces forward mutations and their reversions, and (b) a mutagenic effect which is responsible for the formation and reversion of those mutants which cannot be induced to revert by the base analogs. Inductions of mutations by bromouracil and 2-aminopurine presumably arise by mistakes in base pairing. Freese has pointed out (84) that, whatever the exact mechanism, a purine would be replaced by another purine and a pyrimidine by another pyrimidine. Because of the purine-pyrimidine conservation, the only possible base pair changes that can be induced by such base analogs are the "transitions":

\[
\begin{align*}
A & \leftrightarrow G \\
T & \leftrightarrow C
\end{align*}
\]

(A = adenine, G = guanine, T = thymine, and C = cytosine, or any derivative which is altered at the 5 position). Furthermore, each base analog should be able to induce "transitions" in both directions: in one direction by a pairing mistake made by the base analog when it becomes incorporated into DNA; in the other direction by a pairing mistake which the base analog makes in later replications, after it has been incorporated into DNA.

Proflavine induces mutations of the second kind. Possibly the proflavine-induced mutations involve a molecular transition in which there is a "transversion" of a nucleotide pair in which a purine is replaced by a pyrimidine and vice versa (84):

\[
\begin{align*}
A & \leftrightarrow T \\
T & \leftrightarrow A \\
C & \leftrightarrow G \\
G & \leftrightarrow C
\end{align*}
\]

Rather interesting is a third kind of mutagenic effect, that of a lower pH or higher temperature, which is very likely due to the separation of single purines from the sugar phosphate backbone. The removed purine might be replaced by another (activated) base in the resting DNA, or during DNA replication the new DNA chain, using the altered chain as the complementary template, incorporates a base at the site of the gap which it would not have incorporated normally. If any of the four bases entered this site more or less at random, either molecular "transitions" (I) or "transversions" (II) might result (84). Another possibility is that the deleted base might be skipped during DNA replication so that the
progeny DNA strand would have one less purine at the specific site. The consequences of this event would depend on the nature of the genetic code.

THE DNA OF TUMOR TISSUES

One might postulate that neoplasms differ from normal tissues with respect to DNA structure and composition. However, definitive proof of such differences has not as yet been obtained.

The purine and pyrimidine molar base ratios of normal tissues and tumors are similar (19, 122, 130). In the case of mouse tissues, there is in each case an equivalence of adenine to thymine and guanine to cytosine, with an adenine plus thymine guanine plus cytosine ratio of about 1.25.

Agreement with those of Smith and Kaplan (202) and Prager and Goerner (182) on mouse leukemic tissues, and of Kondo and Osawa (130), who studied the DNA of various normal rat or rabbit tissues. They are at variance, however, with the results of Bendich, Polli, and co-workers (19, 20, 179), who reported chromatographic and physicochemical differences between normal human leukocytes and leukemic cells, and also very marked differences between the DNA chromatographic profiles of rat kidney and brain.

Another approach to the problem is the study of the range of compositional heterogeneity. There is fairly clear-cut evidence that, within a given cell, some DNA molecules contain a relative excess of adenine and thymine and others a relatively greater amount of guanine and cytosine. Doty et al. have shown (66) that the optical density-temperature curves of DNA are characteristic for a given organism. A linear relationship exists between the DNA “melting point” (Tm) and the guanine plus cytosine content. Using this method, we are now engaged in a study of the range of compositional heterogeneity in mouse tissues and tumors.

When dilute solutions of DNA are heated, the optical density remains constant until the ambient temperature is raised to about 80° C. (Chart 1). At higher temperatures, the optical density sharply rises to a value of about 40 per cent above the absorption at room temperature. A very steep optical density-temperature curve is indicative of homogeneity of composition, whereas a broad
curve is suggestive of heterogeneity. This follows from the fact that DNA molecules having a higher proportion of guanine and cytosine "melt" at more elevated temperatures. The exact shape of each curve and the $T_m$ depend not only upon the composition and heterogeneity of the DNA sample but also upon the ionic strength of the DNA solution and the rate of heating at any temperature.

The curves for four tumor DNA samples are illustrated in Chart 1. The $T_m$ values are all about 87.8°C. One-third of the optical density change above and one-third below the $T_m$ take place over a range of about 5.4°C. This is indicative of the same order of magnitude of compositional heterogeneity as was observed by Doty et al. for E. coli DNA (66). It is apparent that, although these four tumors are known to differ greatly in chromosome composition, the range of compositional heterogeneity as well as the average purine and pyrimidine base composition is about the same. It would seem that any differences between them with respect to DNA probably occur at a very subtle level, such as that of the sequence of bases along the polynucleotide chains.

There is one way in which the DNA's of these tumors definitely do differ; that is, in the amount of DNA per cell (122). It is now well established that the DNA content per cell is proportional to the chromosome number. Tetraploid cells contain about twice as much DNA as diploid cells. This is particularly well illustrated when measurements are made on the chromosomes and the DNA of the same cell population (178).

The measurements of the total DNA content have actually strengthened the argument that cancer cells frequently manifest chromosomal abnormalities and hence genetic differences. Recent studies by Ogawa et al. (164) and by Rabotti (184) have confirmed and extended earlier findings by Leuchtenberger, by Bader, and by Atkins and Richards of DNA values in tumors corresponding to aneuploid and polyploid chromosome sets. Rabotti (184) compared the DNA of normal human lymphocytes, primary carcinomas, and their own metastases. Whereas the DNA values of the primary tumors and the normal lymphocytes fell within the range of "diploid" DNA values, the metastases were generally 4N–8N. This seems consistent with the concept of progression in animal tumors already described by Hauschka, Klein, and others.

In many studies of the DNA content of tumors, DNA values corresponding to aneuploid chromosome numbers cannot be distinguished from DNA increases due to premitotic DNA synthesis. For this reason, recent studies by Stich and associates (212) are especially significant. These investigators studied by microspectrophotometric methods the DNA of precancerous and cancerous rat liver cells 2 days after partial hepatectomy. The rats were previously fed $p$-dimethylaminobenzene (DAB) for 5 months. The DNA of the two daughter telophase cells or the metaphase cells were measured. Alterations from genetically homogeneous to genetically heterogeneous populations occurred in the precancerous livers before any definite tumors could be recognized. Hepatoma cells were predominantly subtetraploid in DNA content. Whereas the ratio of DNA in the two normal telophase cells was very close to 1.00, this ratio was often about 0.80 in cells of animals fed DAB for 5 months, indicating clearly that the genetic material was not being equally distributed. Similar results were obtained by Stich and co-workers (213, 214) with human carcinomas.

Aneuploid chromosome and DNA values are indicative of a reorganization of the genetic material in neoplasia. The full significance of the phenomenon becomes apparent when it is recognized that a quantitative relationship exists between the number of replicate genes or chromosomes of a cell and the enzymes or enzyme-forming systems (EFS) of that cell. Changes in the relative number of certain chromosomes and genes compared with others provide a mechanism for irreversibly altering the quantitative balance between anabolic and catabolic processes in the cell. This point will be further elaborated below.

**Gene Catic Level of Control**

Both qualitative and quantitative control of metabolism may be exerted at the genetic level. This is manifest from the changes in the proteins and enzymes of cells which result from: (a) spontaneous or induced gene mutations; (b) gene dosage effects and polyploidy; or (c) the introduction of new genetic material into a cell following bacterial mating, bacteriophage infection, or transformation by DNA solutions.

**Protein alterations.**—Bonner (56, 240) Yanofsky (56, 49), Suskind (220, 221) and others have provided numerous examples of altered proteins resulting from gene mutations. One such example is the enzyme tryptophan synthetase. In mutants, proteins related to this enzyme may be formed; these altered proteins manifest changes in affinity for coenzymes, sensitivity to temperature or to metal inhibitors.

Particularly noteworthy are the experiments of Ingram and associates (112-117) on the hemoglobins. This work has established that a single gene mutation results in the substitution of one
affect one or the other of these genes; for example, for hemoglobin S and C in the identical location. Hemoglobin Da and E close together and those also appears to express itself as an alteration in the hemoglobin S, C, De, and E mutations would be found in two pairs, those for hemoglobin molecules can be formed by the dissociation and recombination in the laboratory of the chains of hemoglobin I with those of hemoglobins S or C.

Human fetal hemoglobin differs from adult hemoglobin in that it has an a chain (as in adult hemoglobin) but also a γ polypeptide chain which differs from the β polypeptide chain of adult hemoglobin. An abnormal fetal hemoglobin (hemoglobin “Barts”) has been observed in an infant in which there are γ chains only (113). It has also been shown that adult hemoglobin H consists solely of β chains. Hemoglobin H is found only in the presence of the gene for thalassemia. The blood picture of infants with hemoglobin “Barts” is like that in thalassemia, but this does not persist into later life, nor do the children develop an abnormal adult hemoglobin. Two interesting hypotheses have been proposed to explain the findings with respect to hemoglobin H and “Barts”: (a) there may be an overproduction of β or γ chains, respectively; or (b) alternatively, the production of the α chains may be inhibited. It would follow from the latter hypothesis that, although α chains of hemoglobins A and F are chemically identical, they are not controlled by the same gene.

Species-specific differences in the amino acid sequence of proteins and peptides of the pituitary gland hormones, cytochrome c, and albumins have also been studied. For recent reviews, see references 17, 72, 108.

Gene dosage effects and polyploidy.—Quantitative control of metabolism is in part effected by the multiplicity of alleles within a cell. This phenomenon may be illustrated by studies of gene dosage in homozygotic and heterozygotic diploid cells or by comparisons of diploid and tetraploid cells.

A number of enzymes and cellular constituents vary with chromosome number in diploid and tetraploid cells (Table 1). For example, both the DNA and the RNA contents per tetraploid lymphoma cells are about twice as great as per diploid lymphoma cells. Likewise, the DNA and the RNA contents of hyperdiploid and hypotetraploid carcinomas vary with the chromosome number (120, 122, 124, 125, 178). Stated differently, the ratio of RNA to DNA is approximately constant for histologically related asynchronously multiplying cells. The ratios for the carcinomas differ, however, from those of the lymphomas.

A number of parameters have been studied by ourselves (126, 127), by Shelton (193), Patterson and Podber (124), and Hausecka and co-workers (97). The following manifest ploidy dependence in diploid and tetraploid carcinoma cells: cell volume, nuclear volume, dry weight, DNA, RNA, histones, nitrogen content, endogenous respiration, glycolysis, transaminase, glucose-6-phosphate dehydrogenase, glycy1-1eucyl peptidase, and the glutathione, aspartate, alanine, and taurine of the free amino acid pool. The following manifest ploidy dependence in diploid and tetraploid lymphoma cells: cell volume, nuclear volume, average nucleoli per cell, dry weight, DNA, RNA, histones, endogenous respiration, glycolysis, succinoxidase, and transaminase.

These relationships are by no means fortuitous. The effects of various modifications of the cellular environment upon metabolism and tumor char-
acteristics have been studied. Respiration, glycolysis, DNA content, and glucose-6-phosphate dehydrogenase activity were not significantly changed by growing the tumor cells: (a) in male rather than female mice; (b) in heterologous strains of mice; or (c) for from 6 to 11 days in vivo prior to harvesting the cells. Moreover, glycolysis or glucose-6-phosphate dehydrogenase were independent of temperature-induced stress of the tumor-bearing mice. It was observed that, if the tumor-bearing mice were kept for ~4 hours at 4°C or overnight at 38°C prior to sacrifice, the tumor cell DNA content, or the constancy of the ratios of glycolysis to DNA or glucose-6-phosphate dehydrogenase to DNA, was not changed by the temperature treatment.

Ethanolamine phosphate content of carcinoma cells were not ploidy-dependent. The glucose-6-phosphate dehydrogenase activity of tetraploid lymphoma cells was about 5-10 times as great as in diploid lymphoma cells. However, the fact that so many parameters do manifest ploidy dependence reinforces the notion that metabolism is quantitatively related to gene dosage and DNA content.

The regulation of respiratory processes may be demonstrated by inhibiting respiration through the addition of glucose or by supplying alternative hydrogen and electron carriers (for example, methylene blue). Inhibition or stimulation from the endogenous level may be elicited with each of the tumor cell strains.

The histologically related tumors did not have euploid multiples of the normal chromosome set. Consequently, an exact quantitative relationship between chromosome number and all cell enzymes was neither anticipated on theoretical grounds nor found experimentally. Cell surface of tetraploid carcinoma cells is only about 1.4 times that of diploid cells (97). Many antigens may be localized on the surface of cells. Tetraploid cells frequently manifest antigenic simplification. The free amino acid content of tissues is related to the genome in an extremely complex fashion. It is therefore not surprising that most of the free amino acids of lymphoma cells or the glutamic acid, glycine, or respiration, and glycolysis are proportional to chromosome number.

Gene dosage effects have been studied in homozygous and heterozygous plants, yeast, Paramecium, and man. The relative activity of invertase in diploid yeast cultures adapted to sucrose is as follows: (a) homozygous recessive, 0; (b) heterozygous, 17; (c) homozygous dominant, 34 (134). In maize, the yellow color of the endosperm is mainly due to zeaxanthin and proportional to the number of Y genes. The same is true of β-carotene (93). Relative anthocyanine and aurone concentrations have been measured in 75 of 27 possible color genotypes of the garden snapdragon, Antirrhinum majus (118). At least three genes, the P, Y, and M genes, control the pigmentation in these plants. The following generalizations may be formulated: (a) Increased numbers of recessive factors result in decreased anthocyanin production; (b) genotypes containing pelargonidin glycoside have a significantly lower
higher proportions of abnormal hemoglobin are a familial characteristic. The presence of the thalassemic abnormal hemoglobin (239, 240). The expression of the abnormal gene is under the modifying influence of other genetic factors, since lower or higher proportions of abnormal hemoglobin are a familial characteristic. The presence of the thalassemia gene exerts a particularly pronounced effect on the expression of the allelic hemoglobin genes. In one case of hemoglobin S-thalassemia disease, 91 per cent hemoglobin S was encountered, while in a child with hemoglobin C-thalassemia disease there was 88 per cent hemoglobin C (156, 199, 240).

Paramecia homzygous recessive for the kappa factor (kk) lack the self-replicating kappa particles in their cytoplasm. Homozygous dominant (KK) individuals of killer stock 51, mating type VII contain 851 particles, whereas type VIII contain only 371 particles. In the heterozygotes (Kk) there are found half as many kappa particles as in the homozygous dominants: 363 particles in mating type VII heterozygotes and 195 in mating type VIII heterozygotes. Moreover, it has been shown that killer animals of type VII liberate at least twice as much paramecin in a given time as killer animals of type VIII (15).

Enzyme changes following bacterial mating, phage infection, or transformation.—E. coli strains containing the i\(+\), x\(+\), y\(+\) genes are inducible for \(\beta\)-galactosidase and permease. Strains having the i\(-\), z\(-\), i\(-\) genes (i.e., \(\beta\)-galactosidase-inducible) can be conjugated with a receptor F\(-\) strain that is genetically z\(+\), i\(+\). Within a few minutes of the time of entry of the z\(+\), i\(+\) genes, which are closely linked, the synthesis of \(\beta\)-galactosidase commences at maximum velocity in the absence of the extracellular inducer (171, 172). The zygotes, therefore, initially act as constitutive organisms. However, after about an hour, the enzyme was no longer formed unless an inducer was added. These experiments will be considered in more detail later.

For the present, it is sufficient to note that in the heteromerozygote, the x\(+\), i\(+\) genes rapidly function in controlling enzyme synthesis.

Pronounced changes in enzyme content also follow within minutes of infection of E. coli with bacteriophage. The following enzymes, which are not normally present in E. coli, are induced following infection with T-even phage: (a) deoxyctydylid acid hydroxymethylase; (b) hydroxymethyl-deoxyctydylid acid kinase; (c) an enzyme for the transfer of glucose from uridine diphospho-glucose directly to the hydroxymethyl cytosine of DNA; and (d) deoxyctydylid acid phosphatase (77-80, 131, 133, 203, 204). The T-even phage contain in their DNA glucosylated hydroxymethyl cytosine in place of cytosine. The deoxyctydylid acid-degrading enzyme may have a dual function in the promotion of the synthesis of phage DNA: (a) It degrades deoxyctydylid triphosphate to a form which is not utilized as a substrate by DNA polymerase; and (b) it provides deoxyctydylid as a substrate for the synthesis of hydroxymethyl-deoxyctydylid acid. Further, it may be a mechanism for the exclusion of phage other than the T-even variety when E. coli undergoes mixed infection with T-even phage and phage which contain cytosine in their DNA.

The following enzymes are greatly increased in activity following infection with T-even phage: (a) thymidylate synthetase; (b) DNA polymerase; and (c) thymidylic acid and deoxyguanylic acid kinases. Thymidylate synthetase activity increases 1000-fold in E. coli B\(\alpha\) after phage infection; this strain is normally deficient in the enzyme (12, 79, 133, 205).

Following infection by T5 phage, there is a 6-10-fold increase in thymidylate synthetase and in thymidylate, deoxyguanylate, and deoxyctydylate kinases but a much smaller increase in deoxyadenylate kinase. There are no changes in any of the kinases when the bacteria are infected with T3 or T7 phage (23). T3 and T7 seem to derive most of their DNA from the host.

New protein synthesis is necessary for the appearance of enzyme activity. Chloramphenicol or amino acid analogs inhibit the emergence of the enzyme activity. Thus, it is unlikely that the enzymes are present in the uninfected cells in a "masked" form. The attractive hypothesis is that the DNA of the phage replace defective genetic material of the bacteria rather than that they merely activate dormant genes or eliminate gene
inhibitors. In support of this notion is the fact that deoxyguanylate kinase of uninfected bacteria differs in certain properties from the enzyme found in T2 infected cells. The enzyme from uninfected bacteria requires the addition of K+ for its activity, but the enzyme from the coliphage-infected cells does not. Although this suggests that the deoxyguanylic acid kinase from the infected cells is a different protein, the results could also be explained by an alteration in the K+ binding strength of the enzyme from infected cells (25).

The acquisition or modification of enzyme activity following infection of animal cells by tumor-inducing viruses has not as yet been extensively studied but remains an attractive possibility.

DNA solutions isolated from wild type strains are capable of transforming mutant strains of Bacillus subtilis to nutritional auxotrophy. Another type of genetic trait which can be transferred by DNA molecules in solution is the capacity to form specific enzymes of the inducible type (206). For example, DNA transformation in Pneumococci causes the formation of an entirely new biosynthetic pathway for the synthesis of specific capsular polysaccharides. In the transformation of the noncapsulated Type III to capsulated Type I, at least three new biochemical systems are formed: (a) oxidation of uridine diphosphoglucone to uridine diphosphogluconic acid, (b) epimerization of uridine diphosphogluconic acid to uridine diphosphogalacturonic acid, (c) polymerization of Type I capsular polysaccharide (9, 206). The following enzymes can be formed in the presence of inducer after transformation by DNA of organisms which were not previously capable of forming these enzymes: (a) mannitol phosphate dehydrogenase; (b) salicin utilization; (c) β-galactosidase; (d) sucrase (206); and (e) alkaline phosphatase (6). It makes no difference whether DNA is isolated from an adapted strain or from a nonadapted strain which has the potential for making enzyme. Replication of the genetically donated DNA can be demonstrated by the isolation of genetically active DNA from transformed organisms. It therefore seems likely that the repair of genetic defects takes place in the DNA of transformed organisms. However, in this as in the other instances discussed in this section, the detailed mechanisms of phenotypic expression remain to be elucidated.

Turnover of enzyme-forming systems (EFS).—Metabolism may be controlled by the number of EFS present at any time. Little is known concerning the biochemical factors which control the rate of production and of degradation of particular EFS. It is reasonable to assume that the activity of the genetic elements in synthesizing given EFS may be modified by inhibitors or activators. The concept has long been popular among embryologists that, although nuclear activity varies in different cells, nuclear potentials remain unchanged (158, 176). The accumulation of certain EFS in the cell cytoplasm may perhaps take place during development and differentiation.

Control at the level of the enzyme-forming system

Enzyme repression and induction.—Let us now consider metabolic control at the level of the EFS. The control of enzyme-protein content by repression and induction are probably examples of control exercised at this level. Enzyme repression is a phenomenon in which the final products of a biosynthetic pathway inhibit in some way the synthesis of the enzymes required to catalyze the first steps of that pathway. A number of examples of repression are known in bacteria (Table 2). For example, the accumulation of arginine within certain bacteria represses the further synthesis of acetylornithinase and ornithine transcarbamylase, enzymes needed for arginine biosynthesis (170, 186).

The mechanism of enzyme repression is of particular significance. Presumably, there is no effect on the formation of the EFS or on its destruction. Rather, the effect is on the action of the EFS (149). When the ornithine transcarbamylase system is released from repression in E. coli, there is evidence of new protein synthesis, but probably no new RNA is made (186).

Pardee, Jacob, and Monod (171) have shown that repression of β-galactosidase activity depends on a dominant gene, i+. Strains of E. coli containing the genes i+, z+ are inducible for β-galactosidase. Those which are genetically i−, z+ are constitutive for the enzyme. It appears that a repressor is produced under the influence of the i+ gene which prevents the synthesis of new enzyme molecules. The constitutive state (i−) results from loss of the capacity to synthesize active repressor. Possibly, the repressor is a nucleic acid (172). The activity of the repressor gene product.

Enzyme repression has not yet been fully explored in animal systems. The repression by creatine of arginine-glycine transamidinase in rats and chicks may be one such example (236). Another apparent example is the repression by glutamic of the glutamine synthetase levels of HeLa cells in tissue culture (62). It would be surprising if many more examples are not encountered. Pos-
Possibly, enzyme repression may in part account for the tissue-specific spectrum of enzymes found in the different tissues of an adult animal. According to this view, the actual enzyme level in a given tissue would depend on the competition between repressors and inducers at any given time. It would in part account for the latent enzymatic potential of a cell.

Many examples of enzyme induction are known in bacteria, and several have been reported in animal tissues (Table 3). These have been particularly studied by Knox and co-workers (128, 129, 142). Of special interest is the fact that thymidine and thymidylate kinase and polymerase may be inducible. The variation of these enzymes in regenerating liver has been studied by Potter, Bol-lum, and co-workers (33, 34) and by Mantasvinos and Canellakis (152). These enzymes appear at the same time (18 hours after the operation) as DNA synthesis in vivo but continue to increase over the period of 18–30 hours while DNA synthesis in vivo is decreasing. When partially hepatectomized rats were irradiated 6–13.5 hours after the operation with 375–1500 r x-rays, the radiation produced essentially complete inhibition of the increase in thymidine kinase and polymerase that would be expected to occur between 6 and 24 hours after partial hepatectomy (18, 32). Recently, Hiatt and Bojarski have shown that, in adult liver or kidney, TMP kinase activity can be markedly increased by thymidine administration. The rise in TMP kinase ordinarily observed in regenerating liver can be prevented by administering fluorouracil, an inhibitor of thymidylate synthesis, thus showing that thymidylate is necessary for the manifestation of the increased activity of thymidylate kinase. However, in thymidine-treated animals, thymidylate kinase activity increases even in the presence of fluorouracil; the increase begins about 1 hour after thymidine administration (105).

Although the experiments of Hiatt and Bojarski are suggestive of enzyme induction, the latter investigators have suggested (107) an alternative explanation for the experimental results. They have shown that: (a) thymidylate kinase activity disappeared from tissue slices incubated in buffer for 3 hours but not from slices incubated with thymidine; (b) a decrease of kinase-specific activity on dilution of liver extract was prevented by

### Table 2

<table>
<thead>
<tr>
<th>Repressor</th>
<th>Enzyme system</th>
<th>Reference</th>
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<tbody>
<tr>
<td>&quot;Arginase&quot;</td>
<td>Acetyl ornithinase, ornithine transcarbamylase, argininosuccinase</td>
<td>90, 170, 186</td>
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<tr>
<td>&quot;Uracil&quot;</td>
<td>Carbamyl aspartate transerase</td>
<td>170</td>
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<td>Products of glucose metabolism</td>
<td>Histidase, uracanase, myoinosital dehydrogenase</td>
<td>149, 161, 162</td>
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<td>&quot;Histidine&quot;</td>
<td>Enzymes of histidine biosynthesis in Salmonella (transaminase, phosphatase, dehydrogenase, dehydrase)</td>
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<tr>
<td>β-galactosidase, permease</td>
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<td>51, 171, 172</td>
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<td>HPO₄</td>
<td>Alkaline phosphatase</td>
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</tr>
<tr>
<td>&quot;Methionine&quot;</td>
<td>Methionine synthetase</td>
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</tr>
<tr>
<td>&quot;Tryptophan&quot;</td>
<td>Tryptophan desmolase</td>
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<td>Tissue differences in animal cells (?)</td>
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### Table 3

<table>
<thead>
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<th>Animal cells</th>
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<td>Enzyme</td>
<td>Reference</td>
</tr>
<tr>
<td>Thymidine kinase (?) (liver)</td>
<td>33, 34, 106, 138</td>
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<tr>
<td>Thymidylate kinase (?) (liver, kidney)</td>
<td>33, 34, 106, 138</td>
</tr>
<tr>
<td>DNA polymerase (?) (liver)</td>
<td>33, 34, 152</td>
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<tr>
<td>Transaminases for phenylalanine, tyrosine, tryptophan (liver)</td>
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</tr>
<tr>
<td>Tryptophan peroxidase-oxidase (liver)</td>
<td>128, 129, 142</td>
</tr>
<tr>
<td>Threonine dehydrase (liver)</td>
<td>191</td>
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the presence of boiled extract, thymidine, or thymidylate in the diluent; and (c) thymidine or thymidylate in the homogenizing medium but not in the assay led to increased activity in normal tissues (107). This suggests that the increase after thymidine is attributable at least in part to enzyme stabilization. Changes in thermal stability of enzymes in the presence of substrate have been encountered by other investigators (91).

Fetal to neonatal life.—Drastic variations are observed in the enzymatic activity of the livers of animals during the transition from fetal to postnatal life. Tyrosine transaminase is absent from the liver of rats and humans at birth but appears within hours after birth (142, 192). Glucose-6-phosphatase, the enzymes of tyrosine oxidation, histidase, tryptophan-peroxidase, phenylalanine-pyruvate and phenylalanine-a-ketoglutarate transaminase, and phenylalanine hydroxylase are other enzymes which are inactive in fetal life but develop postnatally (8, 185). Tyrosine transaminase can be adaptively increased in the livers of adult rats after parenteral administration of tyrosine. Administration of tyrosine to fetal rats in uto failed, however, to elicit an appreciable increase in the low activity of the liver enzyme; the large increase in activity characteristic of adult animals given these agents is not observed until 7 days after birth. When tryptophan was injected into newborn rats during the period when this enzyme was not detected in untreated animals, only small amounts of tryptophan peroxidase-oxidase were synthesized (8). Tryptophan peroxidase-oxidase activity was not found in Novikoff hepatoma or in Chang liver cells grown in tissue culture; both of these tissues were originally derived from liver. Tryptophan administration failed to elicit enzyme activity in these tissues. In the rat, tryptophan injection elicits some activity even at an age when tryptophan peroxidase-oxidase cannot be demonstrated in untreated animals.

Deoxythymidylate deaminase is present in various tumors, rat thymus, embryonic liver, and rat liver which contains proliferating bile duct epithelium, but is absent in normal adult liver and most normal tissues (181). The deaminase is also very active in chick embryonic tissues (150). Thymidylate kinase activity is very high in fetal liver and kidney and declines rapidly after birth to the low levels observed in adult liver (105).

Two attractive working hypotheses may be invoked to explain these findings: (a) increased postnatal synthesis of EFS for the liver enzymes of amino acid metabolism; and (b) enzyme repression of thymidylate kinase or deoxythymidylate deaminase. As further data are reported, it will be possible to assess the validity of these or other hypotheses.

Control at the Enzyme Level

Let us now briefly summarize some of the more familiar examples of metabolic control by activation or inhibition of enzyme activity. Four general categories may be distinguished: (a) control referable to suppressor gene activity; (b) activation related to the dissociation of macromolecules; (c) negative feedback mechanisms; and (d) various miscellaneous factors which affect enzyme kinetics.

Suppressor genes.—Using tryptophan auxotrophs of Neurospora and E. coli, Yanofsky (56, 248, 249), Bonner (36, 63), and Suskind and co-workers (220, 221) have greatly contributed to the clarification of the action of suppressor genes. The growth dependence of certain mutants on tryptophan results from the presence of genetically altered enzymes. One such mutant, strain td-24, requires tryptophan for growth at 25°C, but it grows slowly without tryptophan above 30°C and at this temperature also forms a slight amount of active tryptophan synthetase. In all instances, this mutant forms large quantities of a protein, CRM, which is antigenically related to wild type tryptophan synthetase and which possesses activity in reaction (1) but not reactions (2) or (3):

\[
\begin{align*}
(1) & \quad \text{Indole + triose phosphate} \rightarrow \text{indole glycerol-phosphate} \\
(2) & \quad \text{Indole glycerol phosphate} + \text{serine} \rightarrow \text{tryptophan + triose phosphate} \\
(3) & \quad \text{Indole + serine} \rightarrow \text{tryptophan}
\end{align*}
\]

It has been possible to obtain highly active tryptophan synthetase from this mutant grown at 25°C by a suitable fractionation of crude inactive extracts. An inorganic inhibitor which completely inhibits the fractionated mutant enzyme but which has no effect on the wild type enzyme at comparable concentrations can be isolated from the inactive td-24 preparations. The inhibitor can be found in other td mutants and also in wild type strains. The effect of this inhibitory material can best be duplicated by Zn++. It appears that with strain td-24 gene mutation has resulted in the formation of an altered, metal-sensitive enzyme. The active enzyme which is obtained by the fractionation of crude inactive extracts appears to represent a conversion of CRM to active tryptophan synthetase by the dissociation of a metal-protein complex.

A mutation at a second locus, the suppressor locus, restores the capacity of the mutant, td-24su-24, to grow in the absence of tryptophan. Suskind and Kurek were able to show (221) that...
partially purified preparations of tryptophan synthetase from suppressed mutant strains were still metal-sensitive. Apparently, the suppressor action of the td-24-su-24 strain may be concerned with the control of metal availability and the concentration of metal-binding enzymes.

A second mechanism for suppressor gene activity has been suggested by Glass (87) and others. When there are alternative metabolic pathways for the utilization of common substrates and limitations occur in the availability of the common substrates, the suppression of one pathway may lead to the stimulation of the alternative pathway. This mechanism may apply to the manifold pathways of utilization of tryptophan in Drosophila (87) and to some extent to pigmentation in plants. The chemical effects of three color factors, P, M, and Y, in Antirrhinum majus have been studied quantitatively (118). The P factor controls a special kind of oxidation of the C-3 fragment that joints the two 6-carbon rings of the flavonoid pigments. Anthocyanidins and flavonoids are present only when P is present in the dominant condition (Chart 2). The M factor controls the oxidation of the C-6 ring in the flavones, flavonols, and anthocyanidins; thus the pigments, quercetin, luteolin, and cyanidin, are formed in the presence of M; kampferol, apigenin, and pelargonidin in its absence. The Y factor acts in its dominant form to permit the formation of only small amounts of aurone pigment, and in its recessive form to permit the production of large amounts of aurone. Suppression effects of one gene upon another may be observed suggesting that genes products may compete for a limited source of common precursor. (See also page 1133.)

Chart 2.—Proposed general pathway for flavonoid biosynthesis in A. majus (118)

Dissociation of macromolecules.—E. coli ribosomes (70S particles) may be reversibly dissociated by variations in the pH, ionic strength, or bivalent cation concentrations to 50S and 30S particles (64). Ribonuclease appears to be almost exclusively associated with the 30S components of the 70S particles (74). The ribonuclease activity of the latter becomes active when the structure of the nucleoproteins is disrupted.

An acid phosphatase of rat liver is linked to large particles. The bound enzyme has little or no activity toward added glycercophosphate at pH 6. Aging of the preparations at 0°C, short exposure of the Waring Blender, or repeated freezing and thawing all lead to an irreversible release of the enzyme, which then appears to a large extent in a soluble form. In contrast to the bound form, the free enzyme exhibits a high activity (22).

Ascites tumor cells apparently contain a potent ribonuclease inhibitor. The enzyme of these cells exhibits pH optima at pH's less than 5 and greater than 8, in sharp contrast to that of various normal tissues which had optimal ribonuclease activities within the range, pH 5.6-7.8. In the presence of p-chloromercuribenzoate, a compound which has been shown to reverse the inhibition of ribonuclease by a naturally occurring ribonuclease inhibitor of rat liver, the ascites tumor picture changed completely. The acid and alkaline peaks disappeared and were replaced by a broad maximum between pH 6.5 and 7.2 (54). This suggests that the two optima in the pH activity curves in the absence of p-chloromercuribenzoate were a reflection of the dissociation of the enzyme inhibitor complex at acid and alkaline pH's.

Negative feedback.—Negative feedback represents still another mechanism for controlling enzymatic activity. In this case, a product of a metabolic pathway prevents its own further synthesis, not by inhibiting the EFS as in repression, but by inhibiting the enzyme itself. Several examples of negative feedback inhibition in bacteria are shown in Table 4. To illustrate, threonine inhibits its own biosynthesis by inhibiting homo-
Serine kinase activity (244). l-Isoleucine completely inhibits the action of l-threonine deaminase, the initial enzyme in the sequence leading from l-threonine to l-isoleucine. The apparent affinity of l-threonine deaminase for l-isoleucine is about 100-fold its affinity for l-threonine (234). A negative feedback mechanism for the control of purine biosynthesis has been demonstrated in pigeon liver by Wyngaarden and Ashton (346).

Control of enzyme kinetics.—Many factors for the control of the kinetics of enzymatic reactions are known (170). These include substrate and inhibitor concentrations, salts, metal and coenzyme concentrations, pH, temperature, redox potential, oxygen tension, sulphydryl compounds, the ratio of oxidized and reduced DPN and TPN, the products of enzyme reactions, and the levels of glucose inhibit the respiration of tumor cells (Crabtree Effect). Permeases may control the availability of substrates within a cell. Many hormones probably exert their effects at the enzyme level; for example, epinephrine activates the enzyme, phosphorylase. Virus infection may initiate a series of events which lead to enzyme activation; for example, T-even phage infection seems to activate deoxyribonuclease. The possibility that some viruses modify the activity of the "pace-maker" enzymes of metabolic sequences should receive careful consideration. The stability and turnover of apoenzymes may be altered by the concentrations of available substrate. Finally, enzyme activity could be indirectly modified by the breakdown of cellular compartments.

SYNTHESIS AND PROPERTIES OF THE ENZYME-FORMING SYSTEM

The locus of RNA synthesis.—Let us return to the problem of the synthesis of the EFS. It is attractive to postulate that single-stranded RNA molecules are synthesized at chromosomal sites and then pass to the cytoplasm where they function as components of EFS. There is considerable experimental evidence to support this hypothesis (38, 121, 125), although several questions remain to be answered:

1. Nucleic acid precursors are rapidly incorporated into the RNA of the salivary gland chromosomes of Chironomus and Drosophila larvac (176). In the case of Chironomus, the chromosomal puffs and Balbiani rings were intensely labeled and the nucleoli became labeled first in the neighborhood of the nucleolar organizers and later in the entire nucleus.

2. Radioautographic studies with tritiated cytidine or uridine show that radioactivity is rapidly taken up and concentrated in the RNA of the nucleus of plant cells, Amoeba, Neurospora, tissue culture cells, or ascites tumors in vivo (for reference, see 121, 125). After a brief period of cell incubation, the excess of radioactive precursors may be removed by washing the cells with a medium containing a large "chaser" of nonradioactive uridine or cytidine. The subsequent localization of the label may be followed during continued incubation of the cells. Experiments of this kind show that in the first 10 minutes the nuclear interchromatin areas contain the highest number of silver grains (RNA labeling on the radioautograms), but at later periods the nucleoli are more heavily labeled. Later, the nuclear labeling diminishes, and the radioactivity accumulates in the cytoplasm.

3. Goldstein and Plaut have shown that the transplantation of a radioactive Amoeba nucleus to a nonradioactive Amoeba was followed by the transfer of radioactivity to the host cytoplasm (37, 38).

4. Enucleated fragments of Amoeba fail to incorporate uracil-C14, adenine, or orotic acid into RNA, although incorporation into the RNA of fragments containing the nucleus is rapid. The earlier indications of some synthesis by anucleated fragments can be attributed to the presence of microorganisms in the food vacuoles of the Amoebae (88, 183).

Some adenine turnover in the RNA of enucleated Acetabularia takes place, but there is no net synthesis. Recent experiments indicate that RNA synthesis can take place in the chloroplasts of this alga, but this is at the expense of other cytoplasmic RNA fractions (microsomes, cell sap) which decrease after enucleation (38). The chloroplasts must be viewed as unique cytoplasmic elements, since they are mutable and may be endowed with hereditary characters. Preliminary reports indi-
cate that alga chloroplasts can incorporate the DNA precursor, thymidine, into acid-insoluble linkage (216). Moreover, the mere incorporation of radioactive precursors into RNA in enucleated cells must be regarded with considerable reserve unless it is definitely shown that the incorporation is into the interior of the polynucleotide chains. End-group labeling of "transfer-RNA" may be mistaken for evidence of RNA synthesis (99, 102, 103).

5. Particles morphologically resembling the cytoplasmic ribosomes (8-20 m\(\mu\)) have been observed by Swift in the nucleoli and in the interchromatin areas of rat liver nuclei, whereas somewhat larger particles (30 m\(\mu\)) have been found in the interchromatin areas of nuclei of the pancreas of Triturus (222). Particles 10-30 m\(\mu\) in diameter have also been observed on the lateral loops of lambrush chromosomes and on the chromosomal rings of Balbiani (85). Nucleoprotein particles can be extracted by neutral buffer solutions from isolated nuclei (85). The particles are revealed by electron microscopy to consist of granules of 10-30 m\(\mu\) diameter. In unextracted nuclei, the presence of similar granules may be noted in both the nucleolus and in the extranucleolar regions of the nucleus. The possible relationship of such particles to the ribosomes remains to be elucidated.

6. The nucleoprotein particles which are extracted from the nuclei by neutral saline may be sedimented by centrifuging the extract at 105,000\(\times\) \(g\) for 2 hours in the Spinco preparative centrifuge (85, 121, 125). The particles contain about 50 per cent RNA by weight and are indistinguishable from the microsomes with respect to electrophoretic mobility and nucleotide composition (121, 123, 168). At least one other RNA fraction may be isolated from the nuclei of cells with a molar base composition similar to that of the microsomes (123). High molecular weight RNA is found in the nucleus as well as the cell ribosomes (48).

7. Chemical turnover studies are compatible with the nucleus as a major site of RNA synthesis. Shortly after the injection of labeled RNA precursors into rats or mice, the order of specific activity is as follows: nucleus > soluble cytoplasm > mitochondria-microsomes (13, 57, 58, 154, 155, 201). Of the microsomal subfractions, the ribonucleoprotein granules which are most active in protein synthesis appear to be least active in RNA labeling (36, 194).

The turnover of three nuclear fractions has been measured: (a) the ribonucleoprotein particles which are extracted from the nucleus by neutral saline and sedimented by centrifugation at 105,000\(\times\) \(g\) (Fraction NP); (b) the nonsedimented RNA of this extract (Fraction NSS); and (c) the RNA which is not extracted from the nucleus by this procedure (Fraction R) (85, 125, 168). One hour after the injection of tritiated uridine into ascites tumor-bearing mice, nuclear fraction NP has about 6 times the specific activity of the microsomes, whereas nuclear fraction NSS is also more radioactive than the cytoplasmic supernatant fraction. The residual nuclear RNA (Fraction R) is about 20 times as radioactive as the microsomes. This residual nuclear fraction is presumably associated with the nucleoli and chromosomes.

Cell nuclei contain amino acid-activating enzymes (109, 238) and enzyme systems from the synthesis of proteins (85). The nuclear amino acid-activating enzymes from pig liver readily bind alanine to nuclear soluble RNA from pig liver but are relatively inactive with soluble RNA from all other sources including pig liver cytoplasm (238). Amino acids are also incorporated into the RNA of calf thymus nuclei by "pH 5 enzymes" isolated from the latter source (109).

An alternative method for the study of nuclear RNA synthesis has been suggested by the discovery of Yamana and co-workers (196, 197, 247) and by Georgiev and associates (86) that the extraction of RNA from tissues by aqueous-phenol solutions is incomplete, and that morphologically intact but damaged nuclei persist after such phenol extraction. In this fractionation, possible artifacts arising in the course of the freeze-thawing and homogenizing of tissues may be avoided. Moreover, the tissues are exposed to 45 per cent phenol within minutes of the time that the animals are sacrificed, thereby reducing the problem of the control of endogenous nucleases.

The results of Yamana and co-workers (197) and Georgiev et al. (86) have been confirmed in our laboratory. Table 5 shows the incorporation of tritiated uridine into the various kinds of RNA obtained by the phenol fractionation. Fraction P is the RNA extracted into the aqueous phase by the saline-phenol solution. This fraction is attributable to cytoplasmic RNA and some nuclear RNA. Approximately 90 per cent of the RNA of this fraction may be precipitated with 1 m NaCl. The soluble 10 per cent has a higher specific activity than the RNA precipitable with 1 m NaCl. Fraction D is the precipitate found at the bottom of the tube below the phenol layer. From the interphase between the saline and phenol, the nuclear fraction is obtained. Fraction E is the RNA which is extracted from the nuclei with sodium trichloroacetate, pH 6.9, and phenol. This
fraction also contains most of the DNA of the cell. Fraction R is the residue.

About 80 per cent of the total RNA of the Ehrlich tumor cells was found in Fraction P (Table 5). However, Fraction E had 90 times the specific activity of Fraction P, and well over 90 per cent of the total radioactivity was incorporated into Fraction E plus Fraction R. The RNA of Fraction E is precipitated from solution embedded in fibrous DNA. It is not as yet known whether this represents nascent RNA newly synthesized on the DNA of the chromosomes or not.

### Table 5

<table>
<thead>
<tr>
<th>Fraction*</th>
<th>counts/min</th>
<th>counts/min</th>
<th>Per cent</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg RNA</td>
<td>µg DNA</td>
<td>in RNA</td>
<td>total RNA</td>
</tr>
<tr>
<td>P</td>
<td>1.1</td>
<td>0.5</td>
<td>5.4</td>
<td>80.1</td>
</tr>
<tr>
<td>S</td>
<td>7.6</td>
<td>6.6</td>
<td>0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Nuclear E</td>
<td>93.3</td>
<td>66.7</td>
<td>11.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Nuclear R</td>
<td>74.0</td>
<td>37.5</td>
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<td>1.5</td>
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<tr>
<td>D</td>
<td>2.4</td>
<td>0.2</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Mixed RNA</td>
<td>11.7</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Fraction P extracted from cells with 6 volumes of 0.01 M NaCl, 10^{-3} M phosphate, 10^{-3} M versenate, and 6 volumes of phenol. The RNA is precipitated from the extract by the addition of 2 volumes of ethanol and of potassium acetate to a final concentration of 4%.

Fraction S was precipitated from the supernatant of the above step with 5% trichloroacetic acid.

Nuclear Fraction E is the RNA extracted from the residue of the NaCl-phenol extraction with 5% trichloroacetic acid, pH 6.9, and phenol.

Nuclear fraction R is the residue from the previous step.

Fraction D is a small precipitate formed under the phenol layer.

---

whether it is merely RNA which has aggregated with the DNA. These interesting alternatives are under investigation.

Sibatani et al. (197, 247) have emphasized that the molar base composition of Fraction R differs from that of Fraction P. Differences in the composition of nuclear RNA from cytoplasmic RNA have frequently been reported in the past (57, 58, 75, 108). Such differences could constitute a powerful argument against the role of the nuclear RNA as a precursor of cytoplasmic RNA. It is our view that gross differences in the composition of nuclear and cytoplasmic RNA should be regarded with reserve unless: (a) the nuclear RNA is obtained under conditions where degradation by nucleases is unlikely; and (b) the nuclear RNA can be shown to be free of any DNA. In nucleoprotein preparations containing DNA and RNA in a ratio of 10:1, there is always the possibility that the presence of the DNA distorts the RNA base ratios.

The special case of virus-infected cells requires additional comment. The question is: Are RNA templates synthesized on chromosomal DNA, or may RNA serve as a template for its own replication? Consider an RNA virus. It is known that cell infection by the viral RNA alone is sufficient to initiate viral replication (83). Does a DNA corresponding to the viral RNA preexist in the cell? Or, does the viral RNA act as a template for the synthesis of a new DNA intermediate which might then multiply exponentially to finally synthesize new RNA molecules? The two latter mechanisms could preserve the position of DNA as the only macromolecule capable of reproducing itself from low molecular weight intermediates. On the other hand, if viral RNA can reproduce itself and transfer its information into a form usable by the cell, there would be no reason to assume that some species of cellular RNA could not do the same. This consideration raises the possibility that an independent hereditary system could exist in cells in addition to the one controlled by DNA. If this were the case, an understanding of the mechanisms by which such RNA molecules were replicated could markedly modify our concepts of how DNA is replicated. For example, since RNA is a single-stranded molecule, one might conjecture that the chain is duplicated by identical base pairing rather than complementary base pairing. Or, to preserve the concept of complementary base pairing, one might postulate that the two halves of the chain were complementary to each other or that chains complementary to the viral RNA serve as intermediates in viral RNA synthesis.

Although the answers to these questions are of the utmost importance, few definitive experiments are as yet available. Only two statements can be made with any assurance: (a) new DNA synthesis does not seem to be required for the replication of RNA viruses; and (b) new RNA synthesis seems necessary for the replication of both RNA and DNA viruses (173, 190, 198, 208, 223). Fluorodeoxyuridine or amionopterin, inhibitors of the methylation of deoxyuridylic acid to thymidylic acid and of DNA synthesis, have no effects on either the rate of formation or the yield per cell of the RNA viruses, polio, Newcastle disease virus, and tobacco mosaic virus (190, 198, 208). Bromodeoxyuridine is also ineffective. These antimetabolites do, however, prevent the replication of such DNA viruses as vaccinia and bac.
teriphage. Interference with RNA synthesis shortly after cell infection inhibits the multiplication of bacteriophage, vaccinia, adenovirus, herpes, and influenza virus (173, 233).

Until the question of the replication of RNA viruses is resolved, the problem of the sequence of information transfer in normal cells must be considered as open.

Properties of the EFS.—By the conventional methods of sucrose-CaCl₂ homogenization and differential centrifugation, the following approximate distribution of RNA is found in lymphoma cell fractions: microsomes, 54–61 per cent; mitochondrial-microsomes, 15–20 per cent; cytoplasmic “supernatant,” 5–7 per cent; nuclear “supernatant,” 1–2 per cent; nuclear particles of neutral saline extract, 6–7 per cent; and nuclear residue, 10–14 per cent. The cytoplasmic “supernatant” fraction is unique in that it has a molecular weight of about 40,000, contains the unusual base, 5-ribosyluracil, and functions in the transfer of activated amino acids to the microsomes (52, 98, 99, 111, 119, 251). It is apparent that by either this conventional method of fractionation or by the phenol fractionation (Table 5), most of the cellular RNA is associated with the microsomes. We can arrive at an appreciation of cellular organization by certain calculations and comparisons of animal cell ribosomes with those of plants, bacteria, and yeast.

In exponentially growing bacteria, Tissieres and co-workers (228, 229) found from 80 to 90 per cent of the cell RNA in ribosomes. Ts'o et al. (231–233) found 50–60 per cent of pea seedling RNA in ribosomes. The composition and molecular weight of ribosomes varies with the source and physiological state of the cell (95, 117). Pea seedling, rabbit appendix, and E. coli ribosomes contain about 60 per cent RNA and about 40 per cent protein (35, 228, 229, 231). Rat liver and yeast microsomes contain about 40 per cent RNA (95). The molecular weights of pea seedling, E. coli, yeast, Neurospora and animal ribosomes are about $4-4.5 \times 10^6$, $2.6 \times 10^6$, $4.1 \times 10^6$, and $4.5 \times 10^6$, respectively. These particles manifest sedimentation rates in the ultracentrifuge of about 70S (229) and 75–80S (46, 95, 231). The ribosomal particles may be reversibly dissociated in the absence of Mg⁺⁺ to 30–40S and 40–60S components. These smaller components have approximately the same shape, density, and RNA content as the larger particles. The molar base ratios of the 30S, 50S, and 70S E. coli particles are also quite similar (205). Under certain conditions, two 70–75S particles may combine to form 100–110S particles.

The proteins from purified ribonucleoprotein particles from rat liver contain a high proportion of basic amino acids and only very small amounts of cystine and tryptophan. In this respect and in their low molecular weight, they resemble the histones of the nucleus (43, 55, 250). As in the case of the histones, the basic proteins of the ribosomes are rich in N-terminal proline and alanine groups (43).

It seems probable that the two high molecular weight RNA components with sedimentation constants of about 30S and 18S, respectively, are related to the 30–60S and the 50–40S subunits of the ribosomal particles (94). For the tumors which we have studied, the molecular weights of these RNA components are of the order of magnitude of $2$ and $1 \times 10^6$, respectively. Since we know the total RNA per cell, the per cent RNA in the microsomes, and the approximate molecular weight of the RNA of the ribosomes, we can estimate the number of ribosomes and RNA molecules per cell. Let us assume for the sake of argument that one ribosomal particle with its two RNA components represents one enzyme-forming template and that there are about 2,000 different enzymes in lymphoma cells. We then estimate that there are, on the average, about 2,000 templates for each diploid lymphoma cell enzyme! Tissieres and co-workers (229) have estimated about 90,000 ribosomes per E. coli in the exponential phase of growth, or, perhaps, 45 templates for each enzyme. In the stationary phase, or upon incubation in phosphate buffer, this number decreases by a factor of 4–5 to about 20,000 particles per cell.

It would seem that, compared with E. coli, animal lymphoma cells are extremely well buffered with respect to enzyme-forming systems. Thus, changes in the number of EFS under the influence of the environment or the complete loss of EFS due to genetic alterations must be relatively slow processes.

The time interval which is required to establish a new balance of EFS and enzymes may be designated the phenotypic lag. The factors modifying the phenotypic lag as well as those affecting the induction and selection of cell lines endowed with favorable chromosomal sets are probably important in tumor progression. A cell which has undergone chromosomal changes and gene mutations may still be amenable to control provided that the enzymatic potential of the cell has not been expressed. An understanding of the conditions which facilitate or retard the expression of the cancer phenotypes would undoubtedly be of significance in the control of neoplasia.
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