DEOXYRIBONUCLEIC ACIDS^{1,2}

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The central dogma, that the chromosomal constituent, deoxyribonucleic acid, functions in the storage and transfer of genetic information, developed in part from the classic bacterial transformation experiments of Avery, MacLeod & McCarty (1) [see reference (2) for review of transformation by DNA], the bacteriophage studies of Hershey & Chase (3), and the formulations regarding DNA structure and function by Watson, Crick, Wilkins and their colleagues (4 to 7). The intellectual synthesis achieved has had a profound orienting influence on the multidisciplines comprising biology, as evidenced by the rapidly mushrooming literature dealing with DNA. Only selected aspects of this literature will be treated in this review. The topics discussed will be anchored to the composition and structure of DNA and the effects of various chemical agents in modifying this composition and structure.

It is appropriate, at the outset, to note the increasing number of recent publications demonstrating the genetic effectiveness of purified DNA. As first shown by DiMayorca *et al.* (8), DNA extracted from polyoma infected cells is capable of initiating the formation of intact polyoma virus in mouse embryo cultures and of inducing tumor formation in hamsters (9). The DNA of two additional members of the PAPOVA virus group [rabbit papilloma virus (10, 11, 12), and Simian virus (SV 40) (13)] are also infectious. Virus formation can be initiated in the protoplasts of various *Escherichia coli* strains by purified DNA from at least three bacteriophage, $\phi X174$ (14 to 17), λ phage (18), and phage T1 (19). The DNA of a fourth bacteriophage, SP3, can induce the production of plaque-forming units in *Bacillus subtilis* cultures without recourse to protoplasting provided that the cultures are "competent," that is, susceptible to transformation by DNA (20). Kaiser &

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² The following abbreviations are used: DNase (deoxyribonuclease); RNase (ribonuclease); A, G, C, T, U, I, HMC, \overline{BU} , \overline{IU} , \overline{BC} (adenine, guanine, cytosine, thymine, uracil, hypoxanthine, hydroxymethylcytosine, 5-bromouracil, 5-iodouracil, and 5-bromocytosine); BUDr (5-bromodeoxyuridine); dAT (alternating copolymer of deoxyribonucleotides of A and T, hydrogen bonding not specified); dA \overline{BU} (alternating copolymer of deoxyribonucleotides of A and T, hydrogen bonding not specified); dAT:dAT (hydrogen bonded form of dAT); dAT:dA \overline{BU} (dAT hydrogen bonded to dA \overline{BU}); dGdC (homopolymers of the deoxyribonucleotides of G and \overline{BC}); dI:dC (homopolymers of the deoxyribonucleotides of G and \overline{BC}); dI:dC (homopolymers of the deoxyribonucleotides of I and C, hydrogen bonded together); dI:dBC (homopolymers of the deoxyribonucleotides of I and \overline{BC} , hydrogen bonded together); Tm (melting temperature or midpoint of optical density trar

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Illumina Ex. 2018 IBS v. Illumina IPR2013-00517 Hogness (21) have shown that galactose-negative strains of *E. coli* K12 can be transformed to the galactokinase or galactose transferase positive condition by incubating the bacteria with the DNA of defective transducing phage (λdg) along with ordinary λ phage (which serves as a "helper"). Analysis of the transformed bacteria showed that the active DNA carried phagegenes as well as galactose genes and, in fact, appeared to be the entire chromosome of λ dg. Van de Pol *et al.* (22) have incubated *E. coli* B protoplasts with a T4rII mutant which had been disrupted with urea and with the DNA of wild type T4 phage. They demonstrated that recombination between phage genomes occurred and, hence, that the DNA of wild type T4 phage was biologically active. Finally, auxotrophic strains of *B. subtilis* have been transformed to prototrophy with DNA obtained from purified suspensions of the lysogenic phage, S-1 (23).

PROPERTIES OF DNA

Extraction and deproteinization of DNA.—Although DNA isolation procedures have been greatly improved in recent years, no single method is suitable for the isolation of DNA from all sources. The modifications introduced in classical procedures by Marmur (24) permit the isolation from over 50 different species of microorganisms of a highly polymerized, biologically active product, relatively free from protein and RNA. The organisms are disrupted by lysozyme or detergent treatments, the nucleic acids dissociated from proteins with molar perchlorate, and deproteinized with chloroformisoamyl alcohol. RNA contamination is removed by RNase treatment or by CsCl density gradient centrifugation, and the DNA is selectively precipitated with isopropanol. DNase activity is minimized by the use of chelating agents and by the action of sodium lauryl sulfate.

Various modifications of the phenol-method of Kirby (25, 26) are suitable for the isolation of DNA from viruses (8 to 11, 13, 14, 18, 27) and from animal tissue homogenates without the preliminary preparation of cell nuclear fractions (28 to 33). Aqueous phenol solutions arrest incipient nuclease activity and facilitate the solubilization of proteins. Various anions or combinations of anions are effective in dissociating DNA from protein, particularly those which have the dual properties of interacting with proteins and forming metal complexes (25). The amount of residual protein attached to the DNA depends upon the anion used in conjunction with phenol and the anion concentration. Polysaccharide contamination is eliminated by extraction with 2-methoxyethanol from a potassium phosphate solution of DNA.

Procedures which are satisfactory for the isolation of DNA from most animal tissues yield degraded nonfibrous products from *Drosophila* eggs and fail to liberate DNA in good yield from mammalian sperm or pox viruses. However, by extracting washed eggs with a solution of 0.015M phenolpthalein diphosphate and 0.3M diethyldithiocarbamate and phenol, highly polymerized *Drosophila* DNA may be obtained (34). The difficulties encountered with mammalian sperm probably stem from the inaccessibility of

the DNA due to the nearly impervious keratin-like membrane surrounding the sperm head. However, treatment of the sperm with alcohol-ether to remove lipoproteins from the capsule, followed by extraction with a salinecitrate solution containing 0.25M 2-mercaptoethanol, and finally with trypsin, releases the DNA into solution so that deproteinization can be carried out by the conventional dupanol method (35). The Borenfreund, Fitt & Bendich (35) method has been applied to the problem of deprotenization of pox viruses, whose DNA is also encapsulated in a complex protein coat (36, 37, 38), with the result that double stranded, high molecular weight DNA has been extracted from these viruses with a yield of about 90 to 95 per cent.

How long are DNA molecules?—The molecular weight of DNA, prepared from tissues by the previous methods, generally ranges from $6-14 \times 10^6$. There is now considerable evidence that these DNA preparations consist of fragments of the *in situ* molecules which have undergone enzymatic scission and mechanical breakage during isolation (39 to 43). Long fragile DNA fibers break near the centers when subjected to shearing forces and turbulence, the half molecules then repeat this process, and so on, until some size is reached that will survive the shear being applied. It is not improbable that breakage should produce fairly uniform fragments. DNA is particularly sensitive to shear degradation at the low concentrations which must be used to avoid aggregation (41, 45).

A second problem relates to the evaluation of DNA size by the methods which are currently available. Most of the techniques, such as light scattering, sedimentation velocity, intrinsic viscosity and gradient equilibrium centrifugation, either suffer from defects of principle or practice, or they have not yet been calibrated directly, or checked in the molecular weight range of $30 \text{ to } 300 \times 10^6$ (44).

Chromatographic (39, 40), autoradiographic (46 to 50) and electron micrographic (51 to 53) techniques have proven extremely useful in estimating the molecular size of DNA greater than 107 where other techniques have been found wanting. Employing mild alkali treatment of the virus to release DNA, and versene and extremely dilute solutions to avoid aggregation, Bendet et al. (53) have obtained T3 DNA preparations which in electron micrographs exhibit no branching, are of uniform diameter of about 20 A, and have an average length of 14μ . The DNA of λ phage, when carefully isolated, has a length of about 23 μ (47). All of the genes of phage λ are linearly arranged in a single linkage group. Moreover, a single active unit of λ DNA is infectious (54). The λ DNA has been broken into two halves by controlled hydrodynamic shear and the half molecules separated from the whole molecules by zone sedimentation in a sucrose gradient. One half of the half molecules were capable of transferring genes, s, i, and mi, but not m₆, m₆, or h. Since this incomplete complement is a continuous segment of the λ linkage map, it may be argued that the sequence of genes on the recombination map is the same as the sequence of the nucleotides or blocks of nucleotides which correspond to each of the genes in a molecule of λ DNA (54).

The DNA of T2 and T4 phage have been isolated from the viruses by osmotic shock or the phenol method. For unbroken DNA, the median sedimentation coefficients were 63S and the intrinsic viscosities were about 250 dl/g. The T2 DNA and fragments obtained by shear degradation formed a nearly continuous series whose members fell into a single order whether classified by chromatographic behaviour, sedimentation coefficients, viscosity, or susceptibility to further breakage by hydrodynamic shear (39, 40, 41). To avoid DNA molecular interaction, experiments have been performed at DNA concentrations not greater than 0.4 μ g/ml and the sedimentation behaviour of P³²-labelled T2 or T4 phage DNA has been studied by zone centrifugation through a stabilizing H₂O-D₂O gradient (55). Radioautographic and electron microscopic measurements provide evidence for unbranched thread-like molecules 35 to 52 μ long (46, 48, 49, 51, 55). The results suggest that T2 and T4 DNA consist of one uninterrupted double helix per phage particle, corresponding to a molecular weight of about 130 × 10⁶.

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Genetically, the bacterial nucleus is haploid and the genome comprises a single linkage group that can be transferred entirely as a linear structure at the time of mating (56). The DNA replicates semiconservatively in an orderly fashion, each round of replication being completed before the next starts (57). In short, each bacterial nucleus behaves as if it contained a single molecule of some 4×10^9 molecular weight. Using extraction procedures known to release DNA, but where the explosive forces of turbulence and shear were minimal, Cairns (50) obtained long unbroken threads of *E. coli* DNA; two such threads, both about 400 μ long, were seen at DNA concentrations less than 0.01 μ g/ml. From their length, their estimated molecular weight would be of the order of magnitude of 10⁹. Moreover, Kleinschmidt, Lang & Kahn (52) have produced beautiful electron micrographs showing that protoplasts of *Micrococcus leisodeikticus*, lysed at an air-water interface, release their deoxyribonucleoprotein in the form of a tangled skein that has no visible free ends.

Turning to the DNA of animals, Davison (42) has isolated by plasmolysis and centrifugation fish sperm DNA with unusually high sedimentation coefficients which do not diminish after treatment of the DNA with versene, trypsin, or chymotrypsin, but which are shear sensitive. Unusually high molecular weight DNA has also been isolated from chicken erythrocyte nuclei after the latter were suspended in solutions containing anionic detergents or high salt concentrations (58).

The submicroscopic arrangement of DNA molecules in the sperm of the cave cricket, *Ceuthophilus nigricans*, has been analyzed by highly sensitive polarization optical techniques (59). The results indicate that the DNA molecules are arranged as a coil of a coil in the sperm.

Even the most highly purified DNA preparations contain some residual protein. Hence, the extremely long DNA threads may consist of subunits held together by nonnucleic acid "linkers" (60 to 63). However, the evidence is at present by no means compelling that the residual proteins function in linking nucleic acid subunits. It is debatable whether protein "linkers"

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would increase either the stability or flexibility of DNA threads. Moreover, the presence of "linkers" would impose restrictions on models dealing with the replication and linear assembly of DNA molecules in chromosomes. Until the existence in DNA of nonnucleic acid "linkers" is demonstrated, it is probably legitimate to think of the long DNA threads as single molecules (50, 52, 55).

Nucleotide composition of DNA.—The primary methods for the analysis of DNA nucleotide composition, based upon chromatography or electrophoresis of hydrolytic degradation products, have been supplemented recently by highly accurate and reproducible physicochemical procedures. Of the latter, density gradient centrifugation in CsCl has proven particularly useful since only about 1 to 3 μ g of DNA are required, and information is simultaneously obtained as to DNA strandedness, heterogeneity of composition, bimodal distributions, and the possible occurrence of unusual bases in the DNA (64 to 67). The method is based upon the observation that the buoyant density of DNA in CsCl is directly proportional to its molar per cent guanine plus cytosine content. Denatured DNA behaves in a similar manner, but exhibits a uniformly higher density. The base compositions of the bacteriophage DNA's which contain hydroxymethylcytosine and varied glucose contents are, however, unpredictable from their buoyant densities.

Additional physical methods applicable to the determinations of DNA base compositions include the measurement of the midpoint of the ultraviolet absorbance rise with increasing temperature (68), or the midpoint of the absorbance rise with decreasing pH [pH_{1/2} (69)]. The fact that the ratio of the absorbancies of DNA at 260 and 280 m μ reaches a constant value near pH 3, characteristic of the molar proportion of the bases, may also be used as a basis for a rapid method for estimating base composition (70).

Numerous publications have now appeared providing compilations of the average molar nucleotide compositions of the DNA from at least 73 bacterial species, 14 species of higher plants, 10 species of algae, 4 species of fungi, 18 species of protozoa, 28 species of invertebrates, 31 vertebrate species, 14 bacterial viruses, 13 viruses and rickettsiae of higher animals, 12 insect viruses (67 to 77) and a bacterial episomal factor (78).

Among bacterial species, the mean GC content of DNA varies from 25 to 75 per cent (64, 65, 67, 71, 74). Except for the DNA of *Halobacterium* salinarium, the distribution is unimodal and for a given organism, the heterogeneity of composition, expressed as the standard deviation from the mean, is no greater than 3 mole per cent GC. Bacterial species with mean GC content of DNA differing by 10 per cent probably have few DNA molecules of the same GC content common to the two species. The DNA of *Halobacterium* salinarium, an organism which grows optimally in 25 per cent NaCl, exhibits two DNA bands (each probably double stranded) after CsCl density gradient centrifugation (79). About 20 per cent of the total DNA bands at a position corresponding to 58 per cent GC and the remainder at 67 per cent GC.

Among the algae that have been studied, the diatoms manifest mean

per cent GC values in their DNA of 37 to 40, the green algae 54 to 64, and one brown algae contained 59 (74). The DNA base compositions of several protozoan classes have been measured (75, 76). The flagellate DNA's have 46 to 61 per cent GC, while the ciliate DNA values range from 22 to 35 per cent GC. The GC content of the DNA of the slime mold, *Dictyoselium*, is 22 mole per cent. The value for this organism and that for the DNA of certain *Tetrahymena* species are the lowest thus far encountered from any source when compared with the major components of other organisms. For the ciliates, the range of heterogeneity of composition of DNA molecules, in terms of the per cent GC, was comparable to that found for bacteria and less than that for the DNA of higher organisms (76).

The DNA of higher plants have about 35 to 48 mole per cent GC and often contain relatively high amounts of 5-methylcytosine (67, 77, 80). The DNA of species from the following animal phyla have been measured: porifera, coelenterata, echinodermata, mollusca, arthropoda, annelida, and chordata. The DNA composition of the invertebrate DNA's vary only from 35 to 45 per cent GC (67, 73, 75). The mean nucleotide composition of vertebrate DNA is confined to an even narrower range, 40 to 44 per cent GC (72, 73).

DNA base compositions of the various adult tissues of vertebrate species, and of embryonic and neoplastic tissues do not differ significantly, nor do the DNA preparations differ significantly with respect to heterogeneity of composition (29, 72, 81). The livers from young and old rats have the same DNA base compositions (82). The absence of age specificity with respect to DNA nucleotide composition has also been established for DNA of higher plants (83), and for the DNA of staphylococci 209-R (84).

Through the use of density gradient centrifugation techniques, bimodal DNA distributions have been detected for mouse DNA (29, 71), guinea pig DNA (29), and the DNA of the marine crabs, *Cancer borealis* and *Cancer irroratus* (71). The satellite band of mouse DNA is lighter than the principal DNA band, while the reverse holds for the satellite band of the guinea pig DNA. The minor satellite band of crab DNA is particularly interesting in that it consists of 97 mole per cent dAT:dAT in predominantly alternating sequence (85, 86). The relative amount of the "light DNA" is 30 per cent in *Cancer borealis* and about 10 per cent in *Cancer irroratus*. The biological significance of the satellite DNA bands is not known.

An unusual DNA displaying a molecular weight of about 10,000 is associated with crystalline yeast lactic dehydrogenase. This DNA is not essential for enzyme activity, contains 32.2, 14.9, 17.0, and 35.7 mole per cent adenine, guanine, cytosine and thymine, respectively, and appears to be single stranded. The biological significance of this DNA is also not known (87, 88).

New data have been published on the DNA base composition of several animal viruses. The DNA of the tumor inducing viruses, rabbit papilloma (89), and polyoma (90) contain 50 mole per cent GC, a value corresponding

to the extreme end of the animal host cell distribution, while the DNA of several pox viruses [vaccinia (36, 38), cowpox, rabbit pox, ectromelia (37, 38), and fowl pox (91)], which replicate in the cytoplasm of animal cells, contain about 36 mole per cent GC. The DNA of the cytocytidal nuclear viruses, herpes simplex and pseudorabies, contain 74 mole per cent GC (92), while that of an adenovirus contains about 56 mole per cent GC (93).

The DNA of two recently studied bacterial viruses have unusual properties. In the case of bacteriophage SP8 of *B. subtilis*, 5-hydroxyuracil replaces thymine in the DNA (94). Chemical analyses indicate 43 mole per cent GC, but the Tm and the buoyant density in CsCl yield anomalous values. When the phage DNA is denatured, two bands of relatively equal quantity are observed in the CsCl gradient. The DNA of phage *alpha*, which grows in *Bacillus megatherium*, consists of 41 mole per cent GC, as estimated from the Tm value (95). After denaturation, the DNA of phage *alpha* also manifests two distinct bands on density gradient centrifugation. Possibly, an unknown material, X, is attached to one of the two DNA subunits, causing it to have a heavier buoyant density.

By mating an F⁺ strain of *E. coli* (50 mole per cent GC) with an F⁻ strain of *Serratia marcescens* (58 mole per cent GC), it has been possible to evaluate the base composition of the episomal factor, F-lac (78). Episomally infected *S. marcescens* have in addition to the principal DNA component, a minor band corresponding to 50 mole per cent GC. The minor band represents about one per cent of the total DNA of the recombinant. After fractionating the *S. marcescens* (F-lac) DNA by preparative CsCl density gradient centrifugation, the presence of a second band corresponding to 44 mole per cent GC was observed. This latter band is present to the extent of 0.1 to 0.2 per cent of the total DNA of the sample. The results are consistent with the hypothesis that the genetic component of the episomal element is DNA and with the view that the episomal element is not integrated into the *S. marcescens* genome but rather replicates independently in the cytoplasm or in some unknown association with the host genome.

The differences in the DNA base ratios for different organisms and their homogeneity for the same organism can be explained by evolution from a common organism using the following assumptions: (a) most base pairs in DNA can undergo changes that have no, or only insignificant, selective effect such that organisms experiencing many of them can survive as well as others experiencing few changes; (b) for each DNA species, one kind of base pair, e.g., GC, has been altered more frequently during evaluation than the other one, resulting in a shift of the base ratio (96, 97).

Complementary-RNA.—The facts that proteins are synthesized at ribosomal sites rather than directly on the genes, and that the bulk of the cellular RNA is grossly different in nucleotide composition from the chromosomal DNA, homogeneous in size, and synthesized relatively slowly but nevertheless under genetic control, was in part responsible for the development of the messenger-RNA hypothesis (98, 99). Supporting evidence, showing that cells synthesize metabolically unstable polyribonucleotides with apparent nucleotide compositions which resemble that of DNA (100 to 103), and that the polyribonucleotides differ in sedimentation coefficients from transfer-RNA or ribosomal-RNA but combine reversibly with the ribosomes (99, 104, 105), has greatly stimulated recent research and led to the tentative acceptance of the central messenger-RNA concept. Interestingly enough, however, a more sophisticated second look has been followed by the modification or abandonment of some of the cardinal points on which the messenger-RNA hypothesis was founded.

To illustrate: (a) "Template-RNA" of certain mammalian cells (e.g., reticulocytes) may have a long half life and ribosomal-RNA can be synthesized rapidly in dividing cells (106 to 108). (b) "Pulse labelled-RNA" need not display sedimentation rates lying between 8 to 14S, but may actually be diverse in size and may attain a sedimentation coefficient of 30S (109 to 112). (c) A DNA-independent polymerase, which presumably functions in the synthesis of viral-RNA, accumulates in Mengo virus infected mouse fibroblast cells (113, 114). Moreover, an enzyme system has been described in extracts from *M. lysodeikticus* which utilizes natural and synthetic polyribonucleotides to prime the synthesis of RNA (115). The composition of the primer determines the composition of the product formed. (d) The mechanism for transcribing genetic information from DNA to RNA has not been decisively established. The suggestion has been made that two distinct modes of DNA-dependent RNA synthesis exist for animal and bacterial cells, and that these involve the transient formation of triple helical intermediates (116).

Setting aside interpretive expressions such as informational and messenger-RNA, the following experimental observations should nevertheless be emphasized: (a) a species of newly synthesized polyribonucleotides in T2 or T4 phage infected E. coli cells has been physically separated from the bulk of the cellular RNA, and this species manifests an apparent nucleotide com position resembling that of phage DNA (105) and specifically hybridizes with heat-denatured T2 or T4 DNA (107, 117, 118). This polyribonucleotide species may occur as part of a naturally occurring DNA-RNA complex, may be attached to the ribosomes, or may be found in the soluble-RNA fraction (99, 105, 119, 120). (b) Polyribonucleotides with base compositions corresponding to that of DNA and capable of forming hybrid complexes with denatured DNA also occur in uninfected bacteria (107, 108, 121), and evidence has been presented for their presence in growing (122) or nongrowing yeast cells (123), calf thymus nuclei (106, 124), rat liver and Ehrlich ascites cells (110, 125, 126), plant cells (127 to 129), and Neurospora (130). (c) Bacterial DNA contains a sequence complementary to homologous ribosomal-RNA (131, 132). (d) RNA polymerases have been demonstrated in microbial cells (133 to 137), rat liver nuclei (138), calf thymus nuclei (139), and pea seedlings (140). The enzymes catalyze a DNA-dependent synthesis of RNA in which the product polyribonucleotides resemble the base com-

positions of the double stranded DNA primers. However, when single stranded $\phi X174$ DNA is used as the primer, the product is complementary in base composition to $\phi X174$ DNA (136). The enzymatically synthesized RNA has a nearest neighbor sequence similar to that of the DNA primer (141), and forms specific *in vitro* RNA-DNA hybrid complexes with denatured primer DNA (142). Indeed, both strands of T2 DNA may serve as templates for the enzymatic synthesis of complementary RNA and the polyribonucleotides so synthesized can be self-complementary with a highly ordered, DNA-like secondary structure (143). (e) Virtually all the normal RNA of mammalian and bacterial cells is synthesized by DNA-dependent mechanisms (106, 144 to 146), and (f) a genetic locus regulates bacterial ribosomal-RNA synthesis through unknown mechanisms (147).

DNA fractionation.—Methods for the fractionation of DNA by chromatography on columns of Ecteola-cellulose (148 to 151), calcium phosphate (152 to 154), histone-kieselguhr (155, 156), and methylated serum albuminkieselguhr (27, 28, 85, 157), by paper chromatography (158), by starch zone electrophoresis (159), and by the partition of the DNA in polymer phase systems (160) have been reported. The DNA fractionations described in most of the papers cited above depend upon size differences among the deoxyribonucleotide polymers (85, 158, 160, 161) and hence, are of questionable biological significance. This follows from the fact that the in situ molecular weight of DNA is uncertain and the molecular size distribution of cellular DNA preparations may be an artifact of shear degradation. Nevertheless, chromatography of DNA may be a useful means of removing contaminants or of disaggregating DNA, and may have other important special applications. As in the case of the Ecteola-cellulose exchangers, the methylated-serum albumin-kieselguhr columns fractionate DNA's which differ grossly in molecular size or strandedness. In addition, however, the methylated serum albumin-kieselguhr columns are sensitive to molar nucleotide compositional differences of DNA preparations. Mixtures of B. subtilis (42 per cent GC), S. marcescens (58 per cent GC), and M. leisodeikticus (72 per cent GC) DNA having similar sedimentation coefficients have been partially resolved by Sueoka & Cheng (85). The DNA of greater GC content was eluted at the lower salt concentrations. The naturally occurring dAT: dAT copolymer of the marine crab, Cancer borealis, was isolated uncontaminated by the main DNA component, by chromatography on methylatedserum albumin-kieselguhr columns, whereas efforts to isolate this copolymer by density gradient centrifugation were unsuccessful.

Starch zone electrophoresis has been employed by Matsubara & Takagi (159) to separate relatively large amounts of native DNA from denatured DNA and RNA.

Preparative density gradient centrifugation has proven particularly useful in a number of studies that involve the fractionation of isotopically labelled or biologically active DNA. Taking advantage of the fact that the buoyant density of a DNA molecule depends upon its nucleotide composition, and that the biological activity of transforming principle may be determined after isolation from a CsCl density gradient, Rolfe & Ephrussi-Taylor (162) investigated the nucleotide composition of DNA molecules containing specific genetic markers. They were able to show that the DNA molecules carrying a specific genetic marker: (a) were more homogeneous in buoyant density than the bulk of the DNA; (b) had a mean buoyant density characteristic for a marker, but differing from that of other unlinked markers and from that for the bulk of the DNA; and (c) that the variances of their buoyant density distributions were essentially independent of the marker in question. It thus appears likely that the nucleotide composition of a DNA molecule containing a specific genetic marker is uniquely determined by the compositions of the genes of a definite linkage group comprising this molecule.

Preparative centrifugation in CsCl density gradients has also been employed by Rownd and co-workers (163) to investigate the origin of the residual biological activity of thermally denatured DNA. Studies with denatured *B. subtilis* biologically hybrid DNA have shown that the DNA which bands in the denatured (single stranded) band is biologically inactive and that the residual biological activity is due to species which have not undergone strand separation. The biological activity of the denatured DNA may be restored by renaturation. Renaturation experiments with the denatured transforming DNA also demonstrated that renaturation is not an all-or-none phenomenon, but rather that varying extents of helix reformation are possible.

Nucleotide sequences in DNA.—Information as to the sequence of nucleotides in DNA has been derived from investigations of enzymatic or acid degradation products (77, 80, 164), nearest neighbor sequence studies (165), and experiments on the in vitro formation of renatured DNA hybrids (166). The nature of the enzymatic degradation products is a function of the specificity of the enzyme employed as well as of the structure of the DNA being digested. Acid degradation studies have provided data on the relative frequencies of solitary pyrimidine nucleotides flanked on both sides by purine nucleotides, and on the frequencies of short sequences of pyrimidine nucleotides in the polynucleotide chains. Sequences of three successive deoxycytidylic acids and tracts of four or five successive thymidylic acid residues have been encountered in the DNA of bacterial, plant, and animal origin (77, 80, 164, 167 to 169). There is evidence for a preferential association of methyldeoxycytidylic acid with guanylic acid (77, 80, 170). In mammalian DNA's, methylcytosine was found to occur almost exclusively in the fraction of solitary pyrimidine nucleotides, and in the terminal groups of polypyrimidine sequences carrying monoesterified phosphorus in C-3' of deoxyribose (170). However, in rye germ DNA, dinucleotides of methylcytosine deoxyribonucleotide triphosphate, and dinucleotides of methylcytosine, and cytosine or thymine were also encountered (77, 80). In rye germ DNA fractions prepared by fractional dissociation of nucleoprotein gels, the methylcytosine

content decreased with decreasing molar per cent GC of the DNA. The cytosine and methylcytosine do not exchange positions haphazardly, but the factors controlling this substitution are not understood. Contrary to an early report (171), the replacement of thymine by bromouracil is not associated with gross alterations in *E. coli* DNA nucleotide sequences (172 to 174).

Nearest neighbor or dinucleotide frequency measurements (165) have: (a) confirmed the fact that DNA samples having the same average nucleotide composition have unique and distinctly nonrandom sequence frequencies; (b) provided evidence to support the Watson-Crick model that DNA consists of nucleotide chains of opposite polarities; (c) shown that the dinucleotide frequencies for the DNA's of λ and λ dg bacteriophages and their lysogenic hosts are very similar, and that the values for the T-even phages are virtually indistinguishable from one another but differ from the host cell DNA. However, the sequence frequencies of T1, a phage intermediate between the typically temperate and virulent phages, and T5, a virulent phage in many respects similar to the T-even phages, could be distinguished from one another and from *E. coli* DNA (175). (d) Significant differences have not been detected between the nearest neighbor sequences of the DNA's of several bovine tissues or of four mouse tissues including two tumors (175).

The frequency of a dinucleotide, f_{xy} , may be plotted against the product of the component base frequencies, $f_x f_y$ (176). If the nucleotides were distributed randomly along the chains, f_{xy} would equal $f_x f_y$ and the DNA's of different base compositions would exhibit different dinucleotide frequencies. Although most of the observed sequence frequencies fall within these predictions, several differ sharply. The relation between base and dinucleotide frequency is characteristic for each dinucleotide. Dinucleotide frequencies of animal and plant DNA's differ from those from bacteria. For animal and plant DNA's, the frequencies of GpC come very close to the expectation for randomness but the frequency for the isomeric sequence, CpG, is invariably less than the random value. In bacteria, the reverse of this pattern is found (175, 176). In the case of the TpA and ApT sequence frequencies, bacterial DNA's as well as animal and plant cell DNA's showed a TpA frequency that is decidedly below the prediction for random association; the ApT frequencies, however, are close to the predicted values. The limited data presently available on trinucleotide and longer pyrimidine runs suggest the existence of, at least, second nearest neighbor effects in the formation of pyrimidine sequences in DNA (177).

Thus far the methods for studying the formation of renatured DNA hybrids have not been developed to such a degree that small changes in base composition or base sequences resulting from mutations can be detected. Thus, the DNA from various E. coli strains which vary among themselves by at least several genetic traits, behave in an almost indistinguishable manner when examined for their ability to form *in vitro* hybrids with heavy isotope labelled E. coli B DNA (166). The requirements for *in vitro* DNA hybrid formation among bacterial species are that the heavy and normal DNA

samples have a similar overall base composition and that the bacteria be genetically related when tested by transformation or transduction (166), or in the case of the bacteriophages, by recombination (178). However, microorganisms that can exchange genetic materials by conjugation or F-duction only do not necessarily contain DNA molecules possessing extensive base sequence homologies permitting *in vitro* hybrid formation. Denatured DNA from the T-even phage formed hybrid molecules as did T3 and T7 DNA. However, no indication of homology was found between DNA from T-even and T-odd phage, nor between those and *E. coli* DNA. A hybrid could not be formed between λ phage and unfractionated *E. coli* DNA even though their nearest neighbor sequences are very similar (178).

KIT

Molecular configuration of DNA.-Recent polarized infrared spectra of oriented sheets of Na and Li deoxyribonucleates at various humidities, in both hydrogenated and deuterated states (179), have confirmed the essentials of the Watson-Crick model of DNA configuration (4, 6, 7, 180 to 183), and in addition provided evidence that the configuration applies to the whole of the material and not merely the more crystalline regions. This conclusion is also supported by small angle X-ray scattering data of DNA in solution, which have shown that the weight per unit length was in excellent agreement with the Watson Crick model, was independent of concentration, and was the same in pure water and in saline (184). The hydrodynamic properties of DNA in solution (185 to 188) as well as direct electron microscopic observations (51, 189 to 194) indicate that DNA is a highly extended, stiff coil of moderate permeability and approximately 20A in diameter. The low dichroic ratios obtained with either polarized infrared or ultraviolet irradiation for the in-plane base vibration of the B form of DNA indicate that the base pairs are closely perpendicular to the helix axis. The salts of DNA rapidly exchange D_2O for H_2O showing that the hydrogen atoms that take part in the hydrogen bonding mechanism between the bases are completely accessible to protons and possibly to water molecules (179).

The molecular configurations of the alkali salts of DNA depend upon the alkali metal and the water content. Semicrystalline forms of Na, K, and Rb deoxyribonucleates occur in the B configuration at relative humidities greater than 90 per cent. For Li deoxyribonucleate the B form is crystalline at 66 per cent relative humidity and semicrystalline at 75 to 90 per cent relative humidity. From the point of view of behaviour of solutions of DNA, the semicrystalline B structure is the more significant, since it is the form in which the nucleic acid exists when combined with protamine (195) or histone (196), and also the form stable in the presence of a large excess of water (179). In the B configuration, there are, for each turn of the helix, 10 nucleotide pairs spaced 3.36A apart and almost perpendicular to the fiber axis.

In addition to the B form, a crystalline A form exists for Na deoxyribonucleate at relative humidity between 70 to 80 per cent. In the A form, the bases are tilted by an angle not less than 13° to the normal to the helix axis. For each turn of the helix, there are eleven nucleotide pairs spaced 2.55A

apart. The A form is also found with K and Rb deoxyribonucleates (179). An additional semicrystalline form of Li deoxyribonucleate occurs between 44 per cent and 56 per cent relative humidity (C form) (183). Upon total dehydration, the helical structure of DNA collapses (197).

The polypeptide chains of nucleoprotamine are wrapped around the DNA helix, probably in the shallow groove, in a nearly extended form. The folds occur where nonbasic residues are in pairs. The side chains of the basic residues in the extended part of the polypeptide chain stretch out at right angles to the main chain so that their basic end groups combine with the phosphate groups of DNA. In this structure, the exchangeable hydrogens are not shielded in any way and a very fast deuteration would be expected, as is observed (195, 198, 199).

For deoxyribonucleohistone, which consists of approximately equal amounts of histone and DNA, the B form of DNA persists down to about 80 per cent relative humidity. Infrared, deuteration, and optical rotation studies indicate that over half of the histone is in the α -helical form (196, 199 to 201). Probably, the polypeptide chain of nucleohistone is wound around the DNA in the large groove (198, 200). In vivo, the histone exists as a hydrated protein filling the space between DNA molecules and although associated with the phosphate groups, is not very firmly attached to them (202).

Unlike native, double-stranded DNA, purified $\phi X174$ DNA does not have a rigid fibrillar structure. Instead, like TMV-RNA and denatured bacterial DNA, it appears in electron micrographs as inhomogeneous strands or random coils (203). On the basis of chemical and physicochemical experiments, $\phi X174$ DNA consists essentially of a single stranded chain with a mass corresponding to 5500 nucleotides (204). $\phi X174$ DNA lacks end groups susceptible to hydrolysis by the exonucleases, E. coli phosphodiesterase, venom phosphodiesterase, and spleen phosphodiesterase, results which indicate the unavailability of a free 3'-OH group terminus or a free 5'-OH group terminus. The biological infectivity of the DNA can be thermally inactivated, but this appears to be the result of depurination rather than chain scission. By velocity sedimentation in appropriate solvents, the presence of two discrete components can be demonstrated in preparations of ϕ X174 DNA. It can be shown, either by treatment with pancreatic DNase or by thermal inactivation, that the slower component is the first degradation product, formed by scission of the faster component without significant decrease in molecular weight. The results are consistent with the interpretation that the faster component is a covalently linked ring structure, and the slower component is the open chain degradation product. The infective ϕ X174 DNA chains are chemically and physically indistinguishable from the faster component which has the ring structure (205 to 207).

DNA DENATURATION AND RENATURATION

Conditions affecting denaturation.—For a detailed and critical analysis of DNA denaturation and renaturation, the reader is referred to the recent review by Marmur, Rownd & Schildkraut (188). The salient point which has

emerged during the year is that neither the helix-coil transition (denaturation) nor the reverse (renaturation) is an all or none phenomenon.

Denaturation may be induced by extremes of pH or by heating DNA solutions beyond a critical temperature. The DNA denaturation temperature is very nearly a linear function of the logarithm of the ionic strength (68, 188). At low concentrations of DNA, the molecules are unstable at room temperature when the ionic strength is less than $10^{-4}M$ (208). At room temperature, DNA is stable over the approximate pH range of 2.7 to 12 (69, 209), but denatures at higher or lower pH's. The pH at which DNA denatures depends on the temperature and the ionic strength of the solution.

The stability of DNA secondary structure against temperature or acid denaturation increases with increasing molar per cent GC content (65, 66, 68, 210, 211). The replacement of thymine by \overline{BU} or \overline{IU} also increases the Tm of DNA (212 to 214). In low salts, the $dA\overline{B}\overline{U}$ copolymer is far more stable to heat, but it melts at alkaline pH's at about 1.5 pH units below the dAT copolymer (215 to 218). Conditions can be found where the optical density melting curves for a mixture of dAT and dABU show two clearly resolved steps. The dI:dBC copolymer is also more stable to heat denaturation than the dI: dC copolymer. The stability of the dGd \overline{BC} copolymer is so great that it cannot be melted at neutral pH at the lowest salt concentration studied (219). DeVoe & Tinoco (211) have calculated what they consider the main base-base attractive forces in DNA. Their calculations are consistent with the observed greater Tm of the dABU copolymer as compared with the dAT copolymer. The decreased stability against alkali of the dABU copolymer can be explained by the difference in pK between bromouracil (8.1), and thymine (9.8) (217).

Increasing the ionic strength of the solution enhances the thermal stability of DNA, but the effect tends to level off at high ionic strengths. Some divalent ions at low concentrations (Mg⁺⁺, Ba⁺⁺, Mn⁺⁺, Co⁺⁺, Ni⁺⁺, Zn⁺⁺) increase the Tm of calf thymus DNA, whereas others decrease its Tm [Cu⁺⁺, Cd⁺⁺, Pb⁺⁺ (220, 221)]. The former act by neutralizing the charged phosphate groups and the latter divalent ions are believed to act by breaking the hydrogen bonds by displacing them through coordination to electron donor groups in the nucleotide bases. Mercury ions, by forming reversible complexes with the DNA bases, also cause a small decrease in the denaturation temperature (222 to 225).

Aliphatic diamines have a pronounced effect on the Tm of DNA. The Tm may be increased by 5°C, depending on the chain length and concentration of the amine (226). The extent of protection afforded by the addition of diamines depends on the base composition of the DNA and is directly proportional to the adenine thymine content. The maximal effect is observed with the dAT copolymer (227). Spermine, spermidine, and protamine sulfate also effectively increase the Tm of DNA (228). Spermine affords protection at a concentration of 10^{-6} to $10^{-5}M$, while ten times higher concentrations of spermidine are required to produce the same effect. This has been attributed to the differences in the association constants of the amines with the phosphate groups of DNA (228). The extent of increase in Tm with spermine is a function of the adenine-thymine content of the DNA sample, and is indirectly a linear function of the Tm of the DNA source (229).

Many different organic molecules decrease the stability of the double helical configuration of DNA to varying degrees. These include urea, guanidinium chloride, salicylate (230), formamide (231 to 233), dimethylformamide. dimethylsulfoxide, tetramethylurea (232), formaldehyde (234 to 238), aminonapthol, hydroxylamine (239), and a variety of alcohols (232, 240 to 242). Concentrated solutions of trichloroacetate, trifluoroacetate, thiocyanate, and perchlorate are particularly potent denaturing agents (243, 244). In 7.2M sodium perchlorate, the Tm may be approximately 40° lower than in 0.2M NaCl.

Exposure of DNA to ultraviolet light, X rays, and the photodynamic action of methylene blue lowers the Tm. This is explicable in terms of a weakening of the DNA double helical structure as a result of chemical damage inflicted on the molecule with concomitant localized denaturation along the DNA helix (239, 245, 246). DNA irradiated with ultraviolet light also manifests a shift of the pH to higher values (246).

The stability of the DNA double helix has generally been ascribed to hydrogen bonding. This view seemed logical since agents such as urea, which can compete with water for hydrogen bond formers, were shown to aid in the denaturation of DNA. Moreover, since the stability of the DNA double helix increased with per cent GC content and it had been suggested that GC base pairs could form three hydrogen bonds and AT base pairs only two, hydrogen bonding seemed to account reasonably well for the observed results. However, DeVoe & Tinoco (211) have calculated that dipole-dipole, dipole induced-dipole, and London force interactions among the bases in the helix are large and make the free energy of the helix depend upon base composition and sequence. Their calculations indicate that the hydrogen bond energy of the bases is not the major factor contributing towards holding the strands of the double helix together. Thus, although hydrogen bonds ensure specific base pairing, hydrophobic bonds (247 to 251) may contribute more to the maintenance of DNA secondary structure (227, 232, 243, 244).

A number of anions, which do not themselves react as hydrogen bond donors and acceptors with purines and pyrimidines in aqueous solutions, are nevertheless extremely effective DNA denaturants (243). Trichloroacetate is a much stronger denaturing agent than trifluoroacetate, and acetate is a very poor denaturing agent. Yet the heat of hydrogen bond formation of the corresponding acids (in CCl₄) is all comparable. Thiocyanate is a much stronger denaturant than any of the carboxylate ions. Thiocyanate, trichloroacetate, and perchlorate are more powerful DNA denaturing agents than those prototypical hydrogen bond breakers, urea and guanidinium chloride, and are matched only by certain organic solvents, such as formamide, dimethylformamide, and dimethylsulfoxide (232). Aromaticreagents,

such as phenols and pyridine are especially effective (252). Within a given series, effectiveness shows a parallelism with decrease in ion-solvating power of the reagents as estimated from their effects on the solubility of $Na_{4}P_{2}O_{7}$ in water. There is no correlation with hydrogen bonding ability (243, 244). On the other hand, ethylene glycol-water mixtures are no more effective than methanol-water. The Tm of fully deuterated DNA is essentially identical with that of ordinary DNA in both water or D₂O solutions (227, 253). The differential stability of the GC relative to AT rich DNA's is greater in sodium perchlorate than in dilute saline-citrate solutions and the differential stability is less in methanol-water than in saline-citrate. That the differences in the contributions of AT and GC nucleotide pairs can be varied so greatly. in both directions, by substances that are not, in the usual sense, hydrogen bond breaking agents, strongly suggests that the differences in the stabilities of the AT and GC rich DNA helices do not exclusively arise from hydrogen bond contributions (243, 244). Insofar as the denaturing anions exert their effect on DNA through the modification of the structure of water, they are classed as hydrophobic bond breaking agents.

Partial denaturation.-Most DNA preparations contain a large number of different molecular species, having a range of average base composition representing the various molecular species of the genetic material of the cell and possibly their mechanical breakdown products. Each molecular species in turn has a nonuniform distribution of nucleotide pairs. The breadth of the thermal transition is known to increase as the molecular heterogeneity increases. However, T2 or T4 phage DNA are believed to consist of one molecule and, yet, the thermal denaturation takes place over a range of several degrees of temperature not much narrower than that of bacterial or animal DNA. When T2 or T4 phage DNA are heated at 75° for 3 min in the presence of formaldehyde, the extinction coefficient increases by 25 per cent and the effective buoyant density by about 20 per cent. Nevertheless, electron micrographs indicate that some of the molecules appear to have puddles of denatured polynucleotide chains united to intact segments. Heating to 100° results in the disappearance of the native regions and the only structures then seen are the puddles of polynucleotide chains (254). In 7.2M sodium perchlorate, the transition for even T2 DNA is substantially increased. Moreover, the breadth of the transition does not change over a considerable molecular weight range, and rapid reversibility can be strikingly demonstrated (243).

Studies of the per cent of strand separation of N¹⁵-N¹⁴-hybrid *E. coli* DNA heated at various temperatures in formaldehyde have shown that the process of subunit separation is not directly related to the measured hyperchromicity. No subunits separate until about 75 per cent of full hyperchromicity is obtained, and even at full hyperchromicity, a large fraction of the subunits may be held together (255).

These results can best be explained by the proposal that intramolecular heterogeneity of nucleotide composition broadens the temperature range

over which the helix-coil transition takes place. Regions rich in GC base pairs may be particularly stable. They may contribute negligibly to the hyperchromicity at 259 m μ and may require temperatures above the shoulder of the melting out curve before they dissociate (255). The dependence of optical density on temperature is not necessarily the same at all wave lengths for a given solution. Hence, optical density melting point dispersion curves can be particularly informative (256).

The implication is that large regions of a DNA may be denatured, but that a minimal number of residual ordered regions may persist. In long molecules, a small number of residual nuclei will permit the restacking of extremely long sections of polynucleotide chains and the reestablishment of long-range order.

Additional evidence that partial and reversible denaturation of DNA molecules may occur has been derived from experiments on the reaction of HgCl₂ with DNA, and from studies on the controlled denaturation of animal and bacterial DNA in alcoholic or concentrated perchlorate solutions. Qualitative and quantitative observations of viscosity (222, 223, 257), optical rotatory dispersion (258), measurements of light scattering (259), and ultraviolet absorption (259, 260), indicate that profound alterations in DNA molecular size and shape take place upon the addition of HgCl₂ to DNA solutions. At a HgCl₂ concentration when the ratio of moles of Hg⁺⁺ to moles of nucleotide is 0.5, the reaction product of DNA and Hg⁺⁺ may be described as a two stranded polynucleotide in which nearly all the bases on a given strand are connected to a base on the opposite strand by a Hg⁺⁺ bridge. That the reaction is reversible is shown by the fact that the biological activity of pneumococcal transforming DNA is not impaired by a cycle of mercuration and demercuration (261). With excess Hg⁺⁺, strand separation probably occurs and at a given point on each strand, 1:1 base-mercury complexes are formed (257). At low concentrations, silver ions can also combine with nucleotide bases of the DNA chain, thereby disrupting the hydrogen bonding between strands, but without extensive changes in the shape of the DNA molecule. The reaction can be rapidly and readily reversed by HCN (262).

As judged by the sharp decrease in viscosity, radius of gyration, and specific rotation, and by the ultraviolet absorbance increase, the disruption of DNA secondary structure may be almost complete in methanol-water mixtures. Yet methanol denaturation can be rapidly and readily reversed when the solution is diluted with water (263). Similar partial denaturation may be obtained with higher alcohols and with N,N'-dimethylformamide or tetramethylurea (232). Likewise, the viscosity and specific rotation of DNA are greatly decreased in 7.2M sodium perchlorate at pH 7 and 25°. The secondary structure of DNA can be restored rapidly by reducing the concentration of the anion (243, 244). That the reformation of DNA secondary structure is an intramolecular process can be shown: (a) by the fact that it is not substantially affected by the presence of other DNA molecules, whether these be helical or denatured; (b) by the speed at which secondary structure

is reestablished; and (c) by the fact that it occurs in DNA preparations from viral, bacterial, or animal sources and in solvents varying greatly in ionic strength. With perchlorate, there is a dependence on per cent GC content of DNA, consistent with the concept that GC rich nucleotide pair sequences act as nuclei for the reestablishment of long-range order.

Reversibility of partial denaturation due to crosslinking. Covalent bonds that link the complementary strands of the double helix may be established by treating DNA with sodium nitrite at pH 4.2 (264). When nitrous acid treated DNA is heated for 15 min at 100° in 0.01M NaCl and then quenched in ice, the viscosity decreases only 13 per cent, the buoyant density in CsCl differs from native DNA by less than 0.001 gcm⁻³, and the band widths are almost the same. Unreacted DNA subjected to the same heating and quenching cycle is decreased in viscosity by a factor of ten. It may be estimated that in one hour at 25° and pH 4.2, less than 2 per cent of the amino groups have reacted with sodium nitrite. Also with DNA treated with bis- $(\beta$ -chloroethyl) methylamine-HCl at pH 7, covalent bonds are formed between complementary chains, and the contiguous base pairs are constrained to remain in a small volume element even when the secondary structure is completely disordered. Crosslinking can also be established by irradiating DNA with ultraviolet light (246). When the environmental conditions once more become thermodynamically favorable to the reestablishment of the ordered structure, those nucleotides that have been constrained by a covalent crosslink reassociate in the correct conjugate sequence. By so doing, they reestablish the registration of the rest of the two polynucleotide strands and also provide a nucleus from the ends of which the reformation of the specifically paired double helix may be propagated. The "reversible" DNA made by crosslinking can be made with heterogeneous DNA from animal sources, and not merely from bacterial or viral DNA, reversibility can be displayed in low ionic strength media in which completely denatured T2 DNA "renatures" extremely slowly, if at all, and the reversibility cannot be kinetically blocked (quenched) near 0° (264).

Can the strands be completely separated?—The studies on partial and reversible denaturation of DNA demonstrate that caution is indicated before it is concluded that the DNA duplex has undergone complete strand dissociation after denaturation. Nevertheless, there would appear to be no theoretical considerations precluding 100 per cent rather than 99.9 per cent hydrogen bond dissociation and strand separation, and there are numerous experimental observations suggesting that strand separation has indeed been achieved.

The first convincing demonstration of the separation of polynucleotide strands under denaturing conditions came from the experiments of Meselson & Stahl (57) in which it was shown that N¹⁵-N¹⁴ hybrid DNA could be dissociated by heating into two subunits which banded at characteristic densities after centrifugation in CsCl gradients. The increased band widths of the subunits indicated that the molecular weights had been reduced by half as

compared with the undenatured DNA hybrid. Hybrid DNA molecules can also be denatured to subunits by treatment with 95 per cent formamide for 10 min at 37° (231). DNA denatured by formamide treatment exhibits the usual extensive alterations in physicochemical and optical properties and the loss of transforming activity (231, 233, 265, 266). In formamide solution, DNA was found to have only slightly higher specific rotations and slightly higher absorbancies than its corresponding monomer, and consequently was presumed to be devoid of all formal secondary structure (233). Thermal denaturation in the presence of formaldehyde represents another method for separating DNA hybrids into subunits (238, 267). By reacting with the amino groups of denatured DNA, formaldehyde prevents the polynucleotide chains from reforming intramolecular or intermolecular hydrogen bonds, and the single polynucleotide chains are stabilized (234 to 238, 267). The molecular weight calculated from band variance is just a factor of two lower than that of the undenatured molecules. Formamide denatured transforming DNA can be made to regain its biological activity by removing the formamide, and incubating under conditions where renaturation takes place (231). Likewise, the removal of the formaldehyde groups by dialysis of hydroxymethylated DNA restored conditions for the renaturation of T2 DNA (235).

With the substitution of bromouracil for thymine in DNA, a less alkaline pH is needed to denature the DNA. Shooter & Baldwin (215) have taken advantage of this fact to show that at a pH where heavy DNA (substituted with bromouracil in both subunits) first shows an increase in buoyant density in CsCl corresponding to complete denaturation, the density of hybrid *E. coli* DNA increased by less than one-fifth this increment. Hybrid dAT and $dA\overline{BU}$ copolymers also have been used to study strand disentanglement after melting at an alkaline pH. The results show that these are separate processes, melting being much more rapid when the hybrids are melted in low salt at relatively low temperature (217, 218).

Recent hydrodynamic data obtained by Eigner & Doty (268) support the view that the molecular weight of DNA is halved as a result of thermal denaturation. *E. coli* and *Diplococcus pneumoniae* DNA preparations were heated at a temperature 15° higher than the midpoint of the absorbance transition profile and the intrinsic viscosity and sedimentation coefficient were measured after the heated and rapidly cooled preparations were returned to room temperature. Conditions of low ionic strength and low DNA concentration were selected to obtain denaturation at relatively low Tm and to avoid the complicating effects of interchain aggregation, since this is minimized under conditions where electrostatic repulsion of the charged phosphates of the polynucleotide chains prevent interchain association. Chemical degradation of the polynucleotide chains, an effect known to transpire at higher temperatures (268, 269), was also reduced by working at the relatively lower temperature.

It should be emphasized that the same animal tissue DNA preparations

which display partial and reversible denaturation in alcohol or concentrated anion solutions are also irreversibly denatured at higher concentrations of denaturants and more elevated temperatures. Although DNA denaturation by many organic solvents at 25° could be reversed by water in the presence of electrolyte, denaturation by dimethylsulfoxide was not reversible and the changes in the optical properties in this solvent were relatively larger than in the other nonaqueous solvents (232, 243, 244, 263).

Additional arguments for complete strand separation depend upon the interpretation of experiments on the formation of specific DNA hybrid molecules *in vitro* (renaturation) and the mechanism of DNA replication.

Renaturation.—Renaturation can be distinguished from the reversal of partial denaturation of DNA in that: (a) it occurs only in relatively homogeneous DNA preparations at moderately high ionic strength; (b) is much slower; and (c) it has the properties of an intermolecular process (244, 270, 271). Thermal renaturation takes place with bacteriophage DNA or with the relatively homogeneous bacterial DNA preparations when the DNA is incubated at about 25° below the denaturation temperature (Tm), but does not occur with unfractionated animal DNA or DNA from tobacco leaves. The latter DNA preparations are considerably more heterogeneous with respect to nucleotide composition and sequence (272). It is essential that aggregation be excluded in renaturation experiments by working at low DNA concentrations. Some aggregation most likely takes place during any renaturation; this can be eliminated to a great extent by enzymes that specifically digest denatured DNA, or by exposing renatured DNA to temperatures at or near the Tm in low ionic strength solutions.

Marmur & Lane (270), and Doty *et al.* (273) have shown that renaturation does not occur when the DNA concentration falls below a minimal value, and that renaturation exhibits the properties of a bimolecular reaction. The latter result has, however, been challenged by Cavalieri *et al.* (274) who observed by spectroscopic measurements that the initial kinetics of renaturation of bacterial DNA was first order at 60° and 70°, but that at 80° there was an increasingly second order component resulting in simultaneous unimolecular and bimolecular kinetics. The results were interpreted as due to a zippering-up of a single, denatured but tangled entity and to aggregation by nuclei of hydrogen bonds at the ends of molecules. Additional experiments from other laboratories should help to resolve these discrepancies.

Using T4 DNA labelled with bromouracil, Kozinski & Beer (214) have shown that at low DNA concentrations (less than $1 \mu g/ml$), true hybrids are formed. The renatured molecules are 20A in diameter, have an average length of 10 μ and some cases lengths up to 30 μ , and the expected density in CsCl gradients between light and heavy DNA. End-to-end aggregation was ruled out by sonication experiments. The specific nature of the hydrogen bonding was demonstrated by heating and cooling DNA from different sources (T4 and *H. influenzae*). The two types of DNA banded separately, indicating that there was no entanglement.

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Hybrid double helices have been formed from the alternating DNA copolymers, dAT and dABU, by slowly cooling in solution through the random coil-helix transition in high salt, where both polymers have nearly the same melting temperature. Very high concentrations are needed, in this case, to form hybrids since, at low concentrations, single strands fold back on themselves by means of the alternating sequence instead of uniting with other strands. Formation of hybrid double helices rather than end-to-end aggregates was shown by the failure of sonic breaks to release dAT and dABU from the hybrid and by the existence of a hybrid melting zone when the melting curve was measured in low salt, where the melting zones for dAT: dABU are sharp and separated by 9° (217, 218).

Antibodies against denatured DNA.-Antibodies reacting by C' fixation with denatured DNA or $\phi X174$ DNA have been found in the sera of patients with lupus erythematosus but not in the sera of patients with other diseases or in normal sera. Native double stranded DNA is considerably less reactive (234, 235, 275, 276, 277). Antibodies can also be produced experimentally in rabbits immunized with disorganized T-even bacteriophage (278) but these differ from the antibodies in lupus sera in that the rabbit antibodies react only with DNA isolated from the T-even bacteriophage whereas lupus sera reacted with DNA extracted from a wide variety of sources (277, 278). The determinant for DNA reactive with lupus sera appears principally to be oligonucleotide sequences of thymidylic acid (276). Antibodies with purine specificity have been elicited in rabbits with purinoyl-protein conjugates (279). The antibodies reacted with heat denatured bacterial DNA and ϕ X174 DNA, as judged by complement fixation, and the reaction could be inhibited by various purines and purine derivatives. No complement fixation reaction with rat liver RNA occurred.

DNA REPLICATION AND MUTATIONS

Mechanism of DNA replication.—A number of hypotheses have been put forward as alternatives to the proposal by Watson & Crick (5) that each chain of the DNA duplex serves as a template for the replication of a complementary chain (Semiconservative Replication). Some of the alternative suggestions (62, 280 to 283) stem in part from misgivings concerning the feasibility of unwinding the very long DNA double helices (284, 285). The possibility has therefore been considered that a single deoxyribopolynucleotide chain is synthesized on the DNA double helix, and that the newly synthesized strand then serves as a template for the replication of a complementary chain in a reaction similar to that catalyzed by DNA polymerase. In essence, this would represent "Conservative Replication" of the DNA. Dounce has suggested a mechanism in which the exact copying of one strand of the DNA double helix takes place, perhaps by use of the details of the mechanism proposed in 1952 (286) with the added assumption that some factor prevents the functioning of one of the two strands of the DNA double helix—possibly histone. Cavalieri & Rosenberg (287) visualize a mechanism similar to that occurring when messenger-RNA is synthesized on an undenatured DNA template.

The primary stumbling block to mechanisms positing Conservative Replication is the classic experiment of Meselson & Stahl (57) which clearly shows that the parental DNA molecule is divided equally between two subunits which remain intact through many generations, and that following replication, each daughter molecule receives one parental subunit. By investigating sonic fragments of hybrid C13-N15 DNA, Rolfe (288) has excluded the possibility that the linear subunits are associated end-to-end, instead of side-to-side. The Meselson-Stahl experiment has been amply confirmed using cellular systems other than E. coli (289, 290 to 292) and, in addition, evidence for the Semiconservative replication of the λ phage genome has been obtained by Meselson & Weigle (293), and Kellenberger et al. (294). Although the subunits of Meselson & Stahl have generally been identified with each of the single strands of the DNA double helices, Cavalieri & Rosenberg (280 to 282) have proposed that they are, in fact, double stranded, and that a four stranded entity, held together by biunial bonds, is formed during the replication of parental DNA. The biunial bonds are, however, characterized only as a weak type of connection (281).

In order to test Cavalieri's model, DNA samples of bacterial origin have been studied by small-angle X-ray scattering techniques (295). The experiments have demonstrated that the DNA molecules are rod-like, that the linear mass and the radius of gyration of the rods are in excellent agreement with the Watson-Crick model, and that the molecules are independently distributed in solution, without any side-by-side association. The results were identical for both the unitary and biunial molecules, and apparently did not support Cavalieri's suggestions. The X-ray observations could be reconciled with Cavalieri's experiment, however, on the assumption that the two unitary molecules were loosely associated in the biunial molecule, the average distance between the two subunits being large compared to the diameter. Shooter & Baldwin's experiments (215) are also in apparent contradiction to the Cavalieri model of DNA replication. In a study of the alkaline denaturation of heavy and hybrid bromouracil labelled DNA, Shooter and Baldwin demonstrated that at the pH where heavy DNA first showed that increment in buoyant density corresponding to complete denaturation, the density of the hybrid increased by less than one fifth this increment. If the hybrid had been composed of a biunial molecule (one heavy unitary double helix and one light unitary helix in loose association), a light unitary DNA band should have appeared at the pH at which the heavy unitary molecule denatured.

The hypothesis that the genetic information of bacteriophage DNA is transferred to a protein containing structure shortly after infection has now been abandoned (296, 297). It appears that the protein formed at the initial stages of intracellular growth of a primary T4 phage can also serve for the

replication of the genome of a secondary phage and, hence, that the protein is not individually specific.

The replication of $\phi X174$ DNA represents a special problem. Upon entry into a susceptible cell, single stranded $\phi X174$ DNA is converted to an altered replicative form, which has the properties of double stranded DNA (298). The replicative form, which is infective to bacterial protoplasts, is then multipled many fold. It is not clear whether the replicative form is replicated by a Conservative or Semiconservative mechanism. The manner of formation of the ultimate single stranded progeny from the double stranded replicative form is also obscure. At no time during the infective process is there a pool of free single stranded DNA but, instead, single stranded progeny DNA appear in mature virus particles. The replicative form could either serve as a precursor, one strand being discarded, or as a template for specification of single strands. Failure to find transfer of intact parental DNA to progeny while parental DNA is evidently incorporated into the replicative form suggests that the replicative form serves only as a template for the production of single stranded DNA (298 to 300).

The enzymatic replication of single stranded $\phi X174$ DNA has been studied after limited (20 per cent increase), and extensive (600 per cent) increase (175). Nearest neighbor sequence frequencies obtained under both conditions support a replication mechanism in which a complementary strand to the single stranded primer is first formed and in which both strands are then extensively replicated.

DNA polymerase experiments.—Additional experiments having a bearing on the biological significance of DNA polymerase have been reported. E. coli polymerase catalyzes the synthesis of biologically active DNA (301). This was demonstrated by incubating a purified enzyme with thymidine triphosphate-2-C¹⁴ and a heat denatured D. pneumoniae or B. subtilis primer which contained bromouracil. By density gradient centrifugation, it was shown that the products of incubation included: (a) nonutilized heavy DNA that was not radioactive and contained little transforming activity; (b) hybrid DNA with transforming activity and radioactivity; and (c) light density double stranded DNA lacking bromouracil but containing radioactivity and representing one-third of the total transforming activity. A 20 to 50 per cent net synthesis of DNA was observed in which the maximum transforming activity was 100 times the activity of the denatured B. subtilis primer but about onehalf the activity of undenatured primer.

The nuclei of regenerating rat liver or calf thymus, when prepared in nonaqueous medium by modifications of the Behrens procedure, contain an active DNA polymerase (302). Previously, when homogenates were prepared with aqueous buffers, the enzyme activity had been found only in the "soluble cytoplasmic" cell fraction.

Two factors that may be significant in the regulation of DNA biosynthesis in vivo are: (a) the association and dissociation of the polymerase with the

template; and (b) the level of DNase activity at the site of DNA synthesis. Billen (303, 304) has shown that a polymerase-DNA complex can in fact be extraced from *E. coli* cells. However, quantitative correlations have not as yet been demonstrated. The factor in normal rat liver extracts which inhibits DNA polymerase has been identified by Smellie (302) as a nuclease. Oligonucleotides produced by the action of the liver nuclease on DNA have been isolated and shown to inhibit ascites tumor polymerase activity. Since 3'-phosphoryl terminal oligonucleotides can also inhibit the enzyme because of their inability to accept nucleotide units from the nucleoside triphosphates, it seems likely that 3'-phosphoryl terminal nucleotides are the inhibitors formed as a result of the action of the normal liver nuclease.

In the absence of primer and after a long lag, purified *E. coli* polymerase catalyzes the de novo synthesis of the copolymers of dGdC, dGd \overline{BC} (219), dAT:dAT, and dABU:dABU (174, 305) from the respective triphosphates. The dAT and dGdC copolymers are themselves excellent primers for DNA polymerase. In an unprimed reaction, the rapid synthesis that follows the lag period has the characteristics of autoreplication of the polymers. During the lag period in the unprimed synthesis of dAT and dGdC, there is a progressive increase in priming capacity of the reaction mixture and priming activity is demonstrable within the first fifth of the lag period, and behaves on ultracentrifugation like a large polymer. Probably, the polymer is started by a number of random combinations of nucleotides catalyzed by the enzyme, some product possessing a single and highly regular structure is then favored in further growth, and ordered polymerization continues until the molecule reaches a size suitable for priming. Upon its replication, each of the daughter molecules then becomes a template for further replication. With E. coli DNA polymerase and 5-bromodeoxyuridine triphosphate, the dAT copolymer may be used as a primer for the synthesis of dABU and vice versa (306). Density gradient analyses show that molecules of hybrid density are first synthesized and then a product free from primer. These results contraindicate a disper sive mechanism for replication. If the mechanism of replicating dAT were Conservative, the end results of synthesis would be product, free from primer, and unchanged primer. Existence of a hybrid peak in the density gradient patterns does not rule out a Conservative mechanism since primer and product could be held together at intermediate stages in synthesis and the primer could be reused for further synthesis. Moreover, much of the primer is actually degraded during synthesis. However, optical density melting curves provide decisive evidence that the synthesis follows a Semiconservative mechanism. The fact that hybrid base pairs are formed in the first stage of synthesis is shown by the presence of a hybrid melting zone. Indeed, more than half of the primer is prevented from melting until the hybrid melting zone is reached after only a small amount of dABU synthesis has taken place. The dABU:dABU polymer has a much higher Tm than dAT:dAT, so that a

second step in the melting curves is observed as the $dA\overline{B}\overline{U}$: $dA\overline{B}\overline{U}$ accumulates.

Under the conditions of the enzymatic reaction, the dAT:dAT primer is completely hydrogen bonded. However, the synthesis automatically leads to separation of two newly completed molecules. From the point of view of DNA polymerase, there is no unwinding problem.

DNA and mutations.—Of the common mutagens, bromodeoxyuridine, nitrogen mustards, nitrous acid, and hydroxylamine probably exert their effects by more than one mechanism. An understanding of the mode of action of these agents, as well as the acridines, and ultraviolet light, has advanced considerably during the last year.

Most BUDr induced mutations are probably the result of anomalous base pairing during DNA replication, but these fall into two classes: (a) if an anomalous base pairing with guanine occurs during the incorporation of bromodeoxyuridine triphosphate into DNA, the result after an additional cycle of DNA replication will be the conversion of a GC base pair to an AT base pair; (b) if the anomalous base pairing takes place during the incorpora tion of deoxyguanosine triphosphate into DNA molecules in which bromouracil has been substituted for thymine, an AT base pair would be converted to a GC pair (307 to 313). In addition, radiation sensitizing effects of BUDr (213) and the tendency to induce chromosomal breaks at specific chromosomal localities in mammalian cells (314, 315) should be considered.

The fact that most BUDr induced mutations of T4 bacteriophage can be induced to revert with base analogues supports the concept that they are predominantly base pair transitions (307, 316, 317). Two types of experiments are consistent with the view that either GC to AT or AT to GC transitions can be induced by BUDr: (a) studies on the kinetics of mutation induction by BUDr in T-even phage (308, 309, 318) and bacteria 310 to 313); (b) experiments demonstrating that nitrous acid or hydroxylamine induced phage mutations can be reversed by BUDr and vice versa (319, 320).

Lawley & Brookes (321) have called attention to the fact that ionization of a DNA base, rather than tautomeric change (5), may be a possible factor in mutation. The pKa of BUDr is 8.1 compared with the value of 9.8 for thymidine. Thus, at neutral pH, about 8 per cent of BUDr would exist in the anionic form compared with 0.16 per cent for thymidine. Anomalous base pairing with guanine could therefore occur. Trautner *et al.* (174) have directly demonstrated in experiments with *E. coli* DNA polymerase in which dABU was used as primer, that guanine could be incorporated aberrantly at a frequency of 2000 to 25,000 adenine and thymine nucleotides polymerized. On the other hand, in experiments in which dAT was used as primer, it was shown that an anomalous base pairing between thymine and guanine is very improbable.

The mutagenic actions of nitrous acid and hydroxylamine (320, 322 to

327) have been explained by the deamination of DNA cytosine (319, 328, 329), and the hydrogen bonding properties of the deaminated base with the conclusion that CG base pairs are converted to AT base pairs. The reaction of DNA with nitrous acid may also lead to a deamination of adenine to hypoxanthine with a resulting AT base pair transition to a GC base pair (328). This explanation may be adequate for the induction of point mutations, but it does not anticipate the production of the large chromosomal deletions, which have been observed in T4 phage (330), nor does it account for the mutagenic reactivity of nitrous acid (331), and hydroxylamine (332) for the denatured transforming DNA in contrast to their questionable mutagenicity for native transforming DNA (333). However, crosslinkage of the DNA may result from nitrous acid treatment (264) and the deletion of deaminated cytosine, in some instances, after the reaction with hydroxylamine (319, 329). At the low pH often employed with both reagents, depurination may occur, a process also demonstrated to be mutagenic (307, 324, 334).

Caution is also indicated in interpreting the mechanism of mutagenesis by nitrogen mustards. Difunctional alkylating agents are not necessarily more effective than monofunctional agents as mutagens, but they produce a different mutational spectrum (335). Monofunctional agents alkylate guanine at the N-7 atom while difunctional agents can yield, in addition, di (guanin-7yl) derivatives. If the two alkylated guanine moieties are on opposite strands, crosslinking can occur (264, 335 to 337). Alkylated DNA decomposes with loss of alkylated guanine products and fission of the sugar phosphate chain is thereby potentiated (336, 337). Alkylation of guanine at the N-7 atom can also enhance the acidic ionization of the guanine moieties of DNA, thereby greatly increasing the probability of anomalous base pairing with thymine (338).

The demonstration by Luzzati, Masson & Lerman (339, 340) that the reversible association of proflavine with DNA leads to an intercalation of the acridine dye between nucleotide pair layers in the DNA double helix forcing them 6.8A rather than 3.4A apart, has led to the suggestion (317) that the acridines act as mutagens, not by transversions (307, 316), but because they cause the insertion or the deletion of a base pair. This could lead during information transfer to a break in a polypeptide chain, a considerable alteration of the amino acid sequence, or the production of no protein at all. Mutations of this type would be lethal if they took place at loci controlling the synthesis of proteins of the finished phage. However, base analogue induced mutations presumably permit the synthesis of proteins with only slightly altered amino acid sequences.

It has been shown that base analogues can induce mutants at the "o" locus (resistance to osmotic shock) in phage T4. The "o" gene locus is believed to control a head protein. However, proflavine does not yield "o"

locus mutants (317). Mutations at the "h" locus, another locus which controls a protein of the finished phage, occur spontaneously. Many "h" locus mutants revert with BUDr but not with proflavine. On the other hand, most spontaneous "rII" mutant of T4 phage, which cannot be induced to revert by base analogues, are revertible by acridines. Thus, although the "o" and the "h" loci produce proteins that are part of the finished phage, the "r" locus probably controls proteins that are not part of the finished phage (317, 341).

These experiments have led to the concept that certain back mutations to wild type are not truly to the original wild type but, instead, are additions of one base pair or substractions of another (suppressors) (342). A study of 80 independent mutants at the B cistron of the rII region of phage T4 has provided considerable evidence in support of the deletion-addition hypothesis of acridine mutagenesis. All of the mutants fell within a limited region of the gene and all were non-leaky "r" mutants. All behaved as suppressors of mutant FC O, or suppressors of suppressors, or suppressors of suppressors of suppressors. The proposed explanation is that: (a) addition (deletion) of one base results in a nonfunctional gene because the translation of triplets at the point of base addition (or deletion) will be shifted along one base and will be incorrect; (b) assuming a base has been deleted, a second mutation involving an addition of a nearby base restores function but probably yields an altered protein (pseudowild type); (c) double mutants involving two base deletions lead to absence of gene function; (d) triple mutants in which three bases are deleted (or in which three bases are added) yield pseudowild type; (e) triple mutants with one deletion and two additions or vice versa give loss of gene function. The results are all consistent with a coding ratio of three or a multiple of three (342).

Normally, an alteration confined to the A cistron, which is adjacent to the B cistron, does not prevent the expression of the B cistron, suggesting that there may be a region between the two cistrons which separates them and allows their functions to be expressed individually. Experiments with an rII mutant (r1589) in which the right hand end of the A cistron and part of the left hand end of the B cistron has been deleted (343, 344) have proven highly informative. The A cistron function is lost, but the deletion of the terminal end of the B cistron does not impair the function of the remaining fragment. In mutant r1589, an element is missing and fortuitous joining of the A and B cistron fragments enables continuity to be preserved. Crick et al. (342) have shown that the B cistron function of mutant r1589 can be eliminated by inserting certain deficiencies in its A cistron fragment, while other deficiencies have no effect. The interpretation is that deficiencies corresponding to an integral number of amino acid-determining groups (nucleotide triplets) allows the continued formation of a polypeptide chain while nonintegral deficiencies do not.

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In all organisms so far studied, the incorporation of bromouracil, iodouracil, or chlorouracil in place of thymine into DNA results in sensitization to various types of radiation. This has been observed with BUDr labelled ϕ X174 phage (345), T2 and T4 phage (309, 346), bacteria (347 to 352) and human cells grown in vitro (213, 290, 353). Intracellularly or extracellularly irradiated transforming DNA which contains bromouracil is also increasingly sensitive to UV irradiation (354). BUDr labelled human or bacterial cells and viruses are sensitized not only to UV but also to X rays (290, 350 to 352, 355), to decay of internally incorporated P³² (356) and to light of longer wavelengths emanating from commercial fluorescent fixtures (346). Incorporation of BUDr into the DNA of tissue culture cells also increases chromosomal damage to X irradiation (357). Sensitivity of bacteria to ultraviolet irradiation increases with the extent of incorporation of bromouracil under defined conditions and for a given medium (347). Djordjevic & Szybalski (290) reported that labelling with BUDr of both strands of the DNA double helix was essential for enhancement of ultraviolet sensitivity of mammalian cells. This conclusion has, however, been questioned by Kaplan, Smith & Tomlin (352), who in corresponding experiments with E. coli found that "unifilarlabelled" cells exhibited responses to both X-rays and ultraviolet exposure intermediate between those of controls and "bifilar-labelled" cells.

The ultraviolet and X-ray sensitivity of bacterial cells can be increased by the incorporation of thioguanine into DNA. However, although radiosensitization to X rays is the same in a nitrogen as in an oxygen atmosphere for bromouracil-DNA, the radiosensitization noted under oxygenated conditions with thioguanine (and also mercaptopurine and 2-aminopurine) completely disappeared when X irradiation was carried out in the absence of oxygen (351). On the other hand, bacterial cells in which the thymine of the DNA is partially replaced by azathymine exhibit increased resistance to ultraviolet irradiation (358, 359).

It is probable that much of the deleterious action of ultraviolet arises from photochemical changes in DNA. Hydration of pyrimidines (360, 361) and DNA chain breakage (213, 246) induced by ultraviolet have received considerable attention in the past. Recently, the formation of thymine dimers has aroused increasing interest.

Beukers & Berends first observed (362, 363) that the irradiation of solutions of thymine in the frozen state resulted in a decrease in absorbancy because of the formation of thymine dimers. The assumption that nucleic acids in aqueous solution are surrounded by a highly ordered lattice of water molecules was the reason for this not so obvious experiment. The physical solution around thymine in nucleic acids might be comparable with those in a frozen aqueous solution of this pyrimidine derivative (363). The formation of stable intermolecular crosslinks *invivo* would presumably interfere with DNA replication by preventing strand separation; also, crosslinked bases might display an inability to pair properly during replication. Moreover, the formation of mixed dimers between the thymine of DNA and the uracil of RNA could impair RNA biosynthesis and function (246).

It seemed reasonable to postulate that bromouracil might also undergo dimerization when irradiated with ultraviolet light, and that this might be connected with its radiation sensitizing properties. Although the irradiation of bromouracil in frozen solution did not lead to irradiation products, dimers of bromouracil were in fact formed when frozen solutions of bromouracil and cytosine or bromouracil and uracil were irradiated (364). Frozen solutions of thymidine dinucleotides also yielded photodimerization products (365). However, frozen solutions of azathymine were completely resistant to the photochemical effects of ultraviolet (366).

Thymine dimers were formed when either bacteria (367) or solutions of DNA were irradiated with ultraviolet (368, 369). The bacterial suspensions with fewer survivors contained more thymine dimers (370). When bacteria containing bromouracil in their DNA were irradiated, considerable dehalogenation was observed and the amount of thymine dimer decreased (371); but the rate of the photochemical reaction doubled and a greater number of photochemical products were formed (364). These included the thymine dimer, the bromouracil dimer, and single and double debrominated products.

Although interchain crosslinking occurs and the extent of crosslinking between strands increases as the AT content of the DNA increases, this process cannot account for the ultraviolet inactivation of phage $\phi X174$ (246, 369). However, the presence of two adjacent thymine molecules in a strand of DNA permits formation of intrachain dimers. The importance of intrachain dimers is also apparent from the fact that at equal ultraviolet dose, more thymine is dimerized with denatured single stranded DNA than with double stranded DNA (364, 365, 367, 370, 372).

For frozen solutions of thymine, photodimerization is favored at about 275 m μ but photoreversal to the thymine monomer at 235 m μ (365). The radiation induced reversal to the monomer is also wavelength dependent after the solution of dimer is thawed. At 254 m μ , reversal to free thymine is observed (365). The reversion is at least 10 times more sensitive at 280 m μ than at 313 m μ , and at 360 m μ , no reversal to thymine monomer takes place (368, 373). In an interesting experiment, Setlow & Setlow (374) inactivated the transforming activity of *Hemophilus influenzae* DNA by large doses of 280 m μ radiation. Upon subsequent irradiation at 239 m μ , a considerable proportion of the transforming activity was restored, Approximately half of the inactivation could thus be accounted for by thymine dimer formation.

Photoreactivation by light of wavelengths centering around 370 m μ in the presence of a photoreactivating enzyme constitutes an important mechanism for the repair of ultraviolet damage to living organisms. It now appears that photoreactivation is due to a reconversion of thymine dimer to thymine (246, 369, 375, 376). Whereas samples of irradiated transforming DNA incubated with purified photoreactivation enzyme in the dark and samples incubated with heat inactivated enzyme in the light both showed the same amount of thymine dimer as was present in the untreated irradiated DNA, incubation of irradiated DNA with enzyme in the presence of 370 m μ illumination destroyed over 90 per cent of the dimer present and restored transforming activity.

The photoreactivation enzyme has been demonstrated in yeast and E. coli extracts. However, the species from which transforming DNA is obtained (*H. influenzae, D. pneumoniae, and B. subtilis*) photoreactivate very poorly. Therefore, in experiments in which the photoreactivation of transforming DNA has been studied, partially purified yeast or *E. coli* enzymes have been empolyed. In the dark, the yeast enzyme complexes with DNA (differential centrifugation, Gel filtration on Sephadex G-75). Unirradiated DNA and enzyme when passed together through a column of Sephadex G-75 are slightly separated. Irradiated DNA and enzyme, on the other hand, come through together (375).

For any given survival level, bacteriophage T4 needs about twice the ultraviolet dose as does T2, although the 2 phages contain approximately equal amounts of DNA and do not differ noticeably in their sensitivities to X rays or P³² decay. Ultraviolet sensitivity is controlled by a single gene, u, closely linked to the r17 locus of T2 (346, 377, 378). This gene also controls the extent of PR in such a manner that the greater ultraviolet sensitivity (u⁺ allele) is accompanied by a higher PR, and the smaller ultraviolet sensitivity (u allele) is accompanied by a lower PR. Streisinger (377) designated the u⁺ allele for T2 and the u allele for T4. In this case, u⁺ is the allelic state in which the reactivating action of the u cistron cannot be carried out. By crossing T2 by T4 phage followed by repeated back crossing, a $\overline{T2}u$ strain has been obtained which has half the ultraviolet-sensitivity of ordinary T2 or T2u⁺ phage. The twofold difference in ultraviolet sensitivity of $\overline{T2u}$ and $\overline{T2}u^+$ is due to the fact that when irradiated, both phage incur the same number of ultraviolet hits, but the u-allele is capable of reversing half of these hits following infection. Since the u-allele in one phage particle can perform this function on behalf of all particles in the same cell, one supposes that this gene produces a diffusible enzyme which can repair ultravioletdamaged DNA. Under conditions of photoreactivation, the survival curves of u⁺ and u phage tend to converge. Probably, the actions of the u-geneenzyme and the photoreactivating enzyme overlap. When BUDr replaces thymidine in the phage DNA, the u⁺ phage are about 35 times as sensitive as the control u⁺ stock; the u phage are about 75 times as sensitive as the control u phage. For normal stocks, u and u⁺ survival curves indicate a twofold difference in sensitivity; for the most highly substituted preparations, the u and u⁺ survival curves are indistinguishable (346). It seems probable that the same mechanism for increased sensitivity with BUDr substitution applies

to the cases of ultraviolet and visible light. To explain the results, Stahl *et al.* (346) suggest that thymidine is the component of DNA that incurs u-genereversible and photoreversible damages. Damages in BUDr substituted DNA are neither u-gene reactivable nor photoreversible. It has also been shown by Rupert (375) that *E. coli* DNA in which 35 per cent of the thymidine was substituted by BUDr was not photoreactivable. Also, in BUDr substituted phage T1 and phage P22H5, the UV sensitivity is increased and photoreactivability is lost (379). It would appear that photoreactivation enzymes are inefficient in reversing the bromouracil dimers along a DNA strand (346, 375, 379) or between strands (369).

CHEMICAL MODIFICATIONS OF DNA IN RELATION TO FUNCTION AND CANCER

Histone and meractinomycin complexes.—The concept that histones regulate genetic activity by forming specific complexes with DNA is a venerable one. An interesting experiment supporting this concept has recently been reported (380). It was shown that DNA fully complexed with histone is inactive in support of the DNA-dependent RNA polymerase. The specific activity of the RNA-synthesizing preparation can be increased 1000-fold above that of a crude initial chromatin preparation by purification procedures which remove histones.

The antibiotic, meractinomycin (actinomycin D), is also capable of forming a complex with DNA under in vivo (381 to 383) or in vitro conditions (383 to 385). By doing so, it inhibits the DNA-dependent synthesis of RNA catalyzed by RNA polymerase (385, 386). With the same amount of enzyme which with $2 \mu g/ml$ of meractinomycin produced a 51 per cent inhibition of RNA synthesis, $200 \mu g/ml$ of proflavine or of acriflavine, respectively, caused a 35 per cent and 69 per cent inhibition. The DNA-dependent synthesis of RNA is 10-fold more sensitive to meractinomycin than DNA synthesis (386). In the case of proflavine, enzymatic DNA synthesis is more sensitive than enzymatic RNA formation to the drug, The enzymatic inhibitions by meractinomycin and by proflavine are competitive in nature and can be reversed by increasing concentrations of DNA (386). The biochemical effects resulting from the complexing of DNA by meractinomycin are not only an inhibition of RNA synthesis (144 to 146, 382, 387, 388), but also interference with the final steps in the maturation of viruses (383, 388). Phage DNA is synthesized in meractonimycin treated cells, but, as in the case of acridine treatment (389 to 392), the phage DNA remains sensitive to DNase inactivation and has no protein coat.

Carcinogens and DNA.—The hypothesis that carcinogens exert their effects by modifying DNA structure and function (393) is increasingly supported by recent findings of chemical interactions between cancer inducing agents and DNA. Aside from the nitrogen mustards (335), the potent carcinogens, ethionine (394, 395), dimethylnitrosamine, and diazomethane (396 to 74

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398) alkylate DNA. It has also been shown that 1,2,5,6-dibenzanthracene becomes bound to mouse epidermis DNA (399) and that N-2-fluorenylactamide, when injected into rats, becomes bound to liver nucleic acids (400). The aperature between the DNA backbone chains provides ample space for intercalation of the carcinogen, dibenz (a, j) acridine, which forms soluble complexes with DNA (340). It is tempting to consider the well known requirements of planarity for the carcinogenic activity of polycyclic aromatic hydrocarbons in terms of an intercalated complex with DNA.

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