natures in alkaline solutions to form a double-stranded cyclic coil, a new type of coiled molecule in which all of the turns originally present in the helix are conserved.

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REPLICATION OF NUCLEIC ACIDS

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Several model schemes have been proposed to explain the replication of nucleic acids, in particular the sequence selection of filial nucleotides when a double-stranded nucleic acid undergoes semiconservative replication. (These schemes usually are concerned with a Watson-Crick type DNA, although similar schemes might involve a filial RNA strand.) Two difficulties involved in previously proposed schemes are the unexplained accuracy of replication of nucleotide sequences

and the fact that these models lean too heavily on inadequate mechanical analogies.

In this paper, instead of depending solely on complementarity of hydrogen bonding between purines and pyrmidines for the correct sequence selection of filial nucleotides, we attempt to secure correct replication on the basis of two assumptions: (1) The structure of the parental half of the nucleic acid chain remains almost rigidly preserved throughout the replication process, due to stabilizing molecules located in the DNA grooves, as suggested by Wilkins.[1] (2) We may then assume (*a*) sterical complementarity between the fully structuralized half strand and the incoming filial nucleotides as a sequence determining factor in addition to (*b*) the usual hydrogen bond complementarity condition, and in addition to (*c*) the tendency of the nucleotides of the off-pealing strand to initiate the collection of the correct filial nucleotides due to the specificity of London forces. This assumption gives a triple check on incoming nucleotides.

The hypothesis (1) indeed may imply specialized assumptions[2] about the kind of molecules which fill the DNA grooves. We are fully aware that there are many variants of those assumptions and that they are only subsidiary to the hypothesis (2).

The Intermolecular Forces Relevant for Sequence Selection of Nucleotides.—Complementary base pairing due to hydrogen bond formation has been thoroughly discussed in several papers.[2, 3] Hydrophobic bonds and charge transfer interactions, as important as they are for structural stability of a nucleic acid helix, seem, however, to have little to do with specificity of sequence selection of nucleotides.

Here we will direct special attention to the biological significance of forces due to correlated charge fluctuations, i.e., of interactions essentially of the London-van der Waals type. On the one hand, these interactions account for a general nonspecific attraction of all molecules and therefore tend to give preference to associations between complementary closely fitting structures. Pauling gave quantitative estimates of this effect in connection with various examples from the field of molecular biology.[4] On the other hand, charge fluctuation (London) forces cause a preferential association of identical molecules as nearest neighbors.[5] These interactions become significant when like molecules have been brought close to each other through rapid Brownian motion. If, by that process, two identical molecules approach each other, they will preferentially stay together as long as their corresponding similar net charges, if any, are compensated by gegen-ions.

Specific association of identical molecules by London-van der Waals forces is of biological interest in several connections: on the macromolecular level one may consider the interaction of two identical molecules, each one having many side chains. If two such molecules interact so as to associate many corresponding pairs of identical side chains, the specific interaction energy is additive, and the degree of specificity of attachment becomes extraordinarily high. Such an association may account for the accurate pairing-up of homologous chromosomes in the process of synapsis and inverted synapsis, lining up corresponding genetic loci side-by-side.[6] On a smaller molecular scale, these preferential associations may account for other phenomena of biological specificity.[5-8]

We shall not consider here those replication schemes in which the hydrogen bonds of the parental double-stranded helix remain unopened.[9] We shall restrict our discussion to semiconservative replication of each double-stranded Watson-Crick helix.

Critique of Conventional Replication Schemes.—Conventional replication schemes usually suggest that a lowering of hydrogen ion or other cation concentrations in the surrounding medium leads both to an opening up of the hydrogen bonds between the complementary base pairs and to a decrease in gegen-ion concentration around the phosphates.

The coiling of a strand peeled off from a double-stranded Watson-Crick helix has been postulated and calculated by Longuet-Higgins and Zimm:[10] (1) the transition from the orderly arrangement of nucleotides to random coil single strands implies an entropy gain which derives the reaction in that direction. One should keep in mind, however, that a coiled-up form of nucleic acid is a structural configuration which is not suitable for transmitting information for accurate replication. Instead of such an uncoiling mechanism driven by entropy gain, the uncoiling of the helix may be driven (2) by the energy liberated in the synthesis process.

Another proposal (3) for the opening up of the helix and sequence selection of nucleotides starts from the above suggestion that a change in the ionic surrounding medium occurs which leads to a strong repulsion between the phosphates of the nucleic acid strands. A situation results in which the two opened halves of the helix untwist and pull away in opposite directions, arranging themselves somewhat like the two arms of a Y whose stem represents the double-stranded parental part of the helix. The repeat distance between the phosphates of the opened up strands would be of the order of 7 Å. As the structure of those two branches of the Y is expected to be very different from that of single-stranded halves of an intact Watson-Crick helix, there is not much steric specificity left toward complementary nucleotides, except that provided by the two- or three-point contact between hydrogen bond donors and acceptors on the bases. The specificity implied in a multiple hydrogen bond contact which a sequence of properly stacked bases of a section of one half of a Watson-Crick helix might offer has been lost in the process of opening up.

Whichever of these three mechanisms may be responsible for the untwining of the helix, it is then customarily assumed that complementarity of hydrogen bond pairing possibilities accounts for the correct selection of nucleotides in this semiconservative replication scheme. The limitations of this Watson-Crick argument seem relevant as the double-stranded helix is, in that replication scheme, not firmly structured. Besides, while the helix opens up, H bond complementarity cannot count for much. Donohue, Hotchkiss, and others have also pointed to the possibility of base pairing differing from the traditional Watson-Crick complementarity.[2, 3]

In an effort to design a better scheme to account for the proper sequence selection of filial nucleotides by hydrogen bond complementarity, the suggestion was made that the single-stranded halves of the opened Watson-Crick helix will more reliably direct the semiconservative synthesis of filial nucleic acids if they each faithfully maintain the helical structure which they had in the original helix, thereby providing a lock and key situation. (This scheme was thought to be possible because of the stability which the rapid spinning motion, inherent in the untwisting process, was said to impart to the two halves.)

Considering the possible causes for adequate rigidity of single strands of the opened-up helix, the following comments seem to be in order. (1) The many single bonds along the phosphate-pentose backbone do not provide the desired

rigidity. (2) The hydration of the phosphates of the opened-up strands can scarcely be made responsible for fulfilling that task. (3) The suggestion that the base residues of a single-stranded nucleic acid are so firmly stacked that they provide for the necessary rigidity and for *exact* preservation of the mutual base stacking arrangement right through the separation process is not a tenable one. (4) It has to be recognized that a Y structure with correctly stacked bases is particularly vulnerable at the juncture where a fairly stable double strand separates into two single strands, and yet over this region the greatest stresses occur in holding the long filial arms of the Y to its stem.

To guarantee the preservation of structure of the opened-up strands, enzymes have been invoked in this scheme and analogies have been made which too often resemble textile machinery: guidance devices, properly maintained tension in the threads and supplementary controls have been assumed, explicitly or implicitly.

Proposal of Replication Schemes for Double-Stranded Nucleic Acids.—We would like to comment on possible ways in which structural schemes could satisfactorily describe the process of opening up of a parental nucleic acid double-stranded helix and how molecular interaction could account for the sequence selection of filial nucleotides. We will focus our attention on double-strand replication schemes which indeed do provide for more reliable information transfer than do single-strand primer situations.

Wilkins discussed the incorporation of a single-stranded protein chain into one of the grooves of the Watson-Crick helix, with the phosphates along each strand of that helix spaced about 7 Å apart. We shall call such an arrangement a Watson-Crick-Wilkins helix (cf. Fig. 1). A protein lying within the groove of such a helix, with its side chains reaching alternately in opposite directions, will easily be able to adjust its peptides to a repeat distance of 7 Å, thus enabling every side chain to reach toward every phosphate of the nucleic acid helix strands, respectively. Wilkins also considered the insertion of two protein chains into the two grooves of a double-stranded nucleic acid helix (Fig. 1). We do not want to specify in detail what role the protein chains laid into nucleic acid grooves might play in terms of functioning as an enzyme—perhaps as a synthesizing enzyme—but this is obviously an important issue. The presence of protein chains in the grooves of some nucleic acids is indicated in Wilkins' experiments; so far, there is no experimental evidence for or against the existence of a similar nucleoprotein association during the process of nucleic acid synthesis.

The manner in which the two groove-filling strands attach themselves to the Watson-Crick structure depends also on the $C_2'H$ in the narrow groove of DNA (or $C_2'OH$ in RNA). With regard to the arrangement of the molecular strands which are assumed to fill the grooves of the Watson-Crick-Wilkins helix, a variety of possibilities exist.

Let us assume both of the grooves to be filled. These protein molecules adjusted to the 7 Å repeat distance would fill the grooves tightly. These proteins would be sterically complementary to the grooves of the nucleic acids and be specific toward them. This entire structure is very compact indeed, the amino acid side chains binding the structure together like fibers oriented along lines arranged in left-handed helix direction (Fig. 1). The stabilizing influence of the proteins is important. Instead of protein chains, single-stranded nucleic acid chains might be

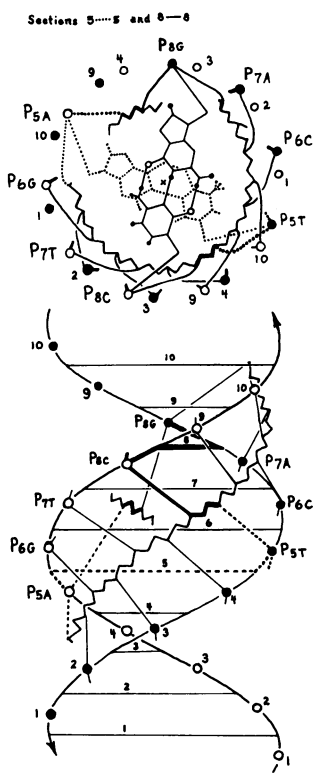

FIG. 1.—Side view (lower diagram) and cross section 5–5 and 8–8 through lower diagram (upper diagram) of a Watson-Crick-Wilkins helix, using the Wilkins-Langridge B structure of DNA.[13] The location of the phosphates is indicated by large dots along one helical chain and small circles along the other. The fifth through the eighth base layers have their phosphates marked P and a letter referring to its attached base. Those layers are principally involved in the stage of replication shown in the following two figures. The pentoses are not specially marked. The two grooves of the nucleic acid are filled with two protein chains, whose backbones are indicated by zigzag lines with the side chains drawn as lines reaching from the protein backbone to the phosphates. For easier legibility, the protein in the wider groove is indicated by a short zigzag line with only the two side chains reaching to the phosphates P_{5A} and P_{8G}. This makes the subunit 5, 6, 7, 8, very stable and compact, similarly for 6, 7, 8, 9, etc. The narrow groove protein has two corresponding side chains reaching to the P_{5T} and P_{8C}. The top picture shows a cross section of the intact Watson-Crick-Wilkins helix with base pair layer 5 dotted and 8 heavily drawn.

laid into one or both grooves of the nucleic acid double-stranded helix. The bases of those chains reach alternately to the phosphates of one strand and to those of the other strand of the Watson-Crick helix. This would amount to a steric arrangement similar to the one just discussed. Those groove-inhabiting nucleic acid strands might even be in the form of several shorter pieces of nucleic acid of a nonspecific type and would, of course, be much more bulky than the proteins.

In either case, the strength of the attachment of the bridges to the phosphates may be considerable. One can easily imagine a change of ionic conditions in the medium which would cause the structure to become unstable and the double-stranded nucleic acid helix to be pried open (cf. upper part, Fig. 2). This may be accomplished by the breaking loose of the rudimentarily indicated second protein chain from the phosphate P_{8G}.

An attractive trait of the scheme of Figures 1 and 2 is that the parental nucleic acid is held firmly together throughout the process of filial helix formation and that the proteins supply the necessary support.

Sequence Selection of Filial Nucleotides.—We will now consider whether the semiconservative synthesis of a nucleic acid molecule may occur by a scheme in which charge fluctuation interactions (c) provide for the primary selection of the appropriate nucletide sequence, a sequence of filial nucleotides identical with those on one of the parental strands which peels off in the process.

In addition to the specificity of those London-van der Waals interactions, the complementary hydrogen bond association (b) between adenine and thymine (or

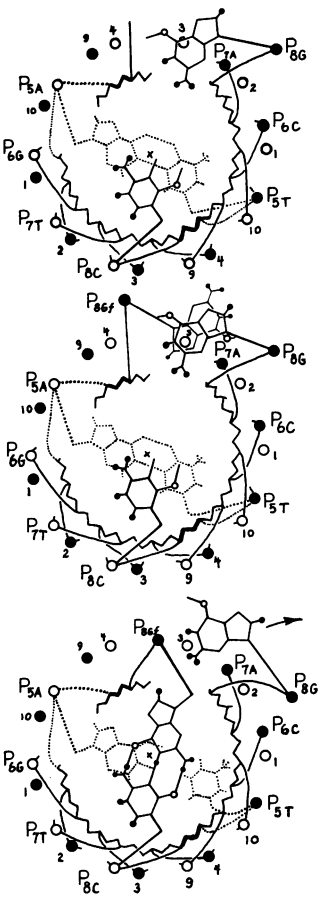

FIG. 2.—These figures refer to our main proposal for nucleic acid replication of the semiconservative type. The process starts with the intact double-stranded Watson-Crick-Wilkins helical structure pictured in Fig. 1. A change in the ionic condition of the medium looses the hydrogen bonding between complementary bases and makes the P_{8G} phosphate break loose from the wide-groove protein side chain (the straight vertical line on the upper part of top of Fig. 2), the guanine 8G thereupon being pried out. A filial guanine $8G_f$ (f standing for filial), brought on by Brownian motion and specifically held by London forces to guanine 8G, attaches itself to the wide groove protein side chain at P_{8Gf} (middle of Fig. 2) and thereafter snugly fits into the open slot (bottom of Fig. 2); the process repeats itself at the next and lower levels. Whereas during replication the scarcity of protons lessens the influence of hydrogen bonding, the re-establishment of normal ionic conditions thereafter brings the Watson-Crick complementarity condition in force again. The nucleotide 8G is a monomer of one of the parental nucleic acid strands which peels off eventually. This single-strand coil may pair with another single strand. Figure 2 is drawn so that the conservative half of the nucleic acid and the two groove proteins do not lose their shape. This was necessary to make the drawing readable.

uracil) and between guanine and cytosine is, of course, a condition which should be satisfied in the subsequent formation of the Watson-Crick DNA (or similar RNA or hybrid DNA-RNA) helixes. Hydrogen bond complementarity would be considered as but a final condition to be satisfied by newly synthesized chains of nucleic acid. The satisfaction of this complementarity based on Chargaff's rule is presumably a necessary condition for orderly helix formation, but is open to question if taken as a sufficient condition.

The proper selection of the correct filial nucleotide sequence may also take advantage of the steric complementarity (a) of the two protein chains with the grooves of the Watson-Crick double-stranded helix. This complementarity may aid in permitting only proper filial nucleotides to enter the newly formed semiconserved nucleic acid structure. A structural illustration of this scheme is given in Figures 1 and 2.

When the double-stranded helix is pried open, nucleotides (e.g., 8G) snap out, one by one, and thus are exposed to the surrounding medium. With previously released nucleotides they form a single strand which eventually peels off.

Correct replication is considered to be initiated by a selection of a sequence of "filial" nucleotides from the pool of activated nucleotides in the medium. Brown-

ian motion moves these nucleotides around in a random fashion and with a speed which at molecular dimensions is exceedingly high. Once a filial nucleotide has reached an identical partner on the parental chain of exposed nucleotides, it will preferentially stay there and be properly oriented, lying side-by-side with its partner due to the specificity of London forces (center of Fig. 2). This attractive force is effective if the static electric repulsion between the identical molecule pairs is compensated by sufficient numbers of gegen-ions from the medium. This neutralization of static charges and dipole, etc., moments is assumed to be achieved at the time of sequence selection of filial nucleotides, whereas at other times in the cycle charge complementarity such as evidenced in specific hydrogen bonding and other specific electrostatic base associations may come to the foreground.

In the particular scheme presented in Figure 2, an appropriate specific nucleotide destined for the filial chain, after being collected by specific London forces, attaches itself to the free amino acid side chain at the location P_{8Gf}· (center, Fig. 2). Being attached to only one amino acid side chain, the "filial" nucleotide is not sterically restrained from fitting into the cavity formerly occupied by the parental nucleotide 8G (lower part, Fig. 2).

Once the proper filial nucleotides from the medium have been collected and fall snugly in the place formerly occupied by the parental bases, and when normal

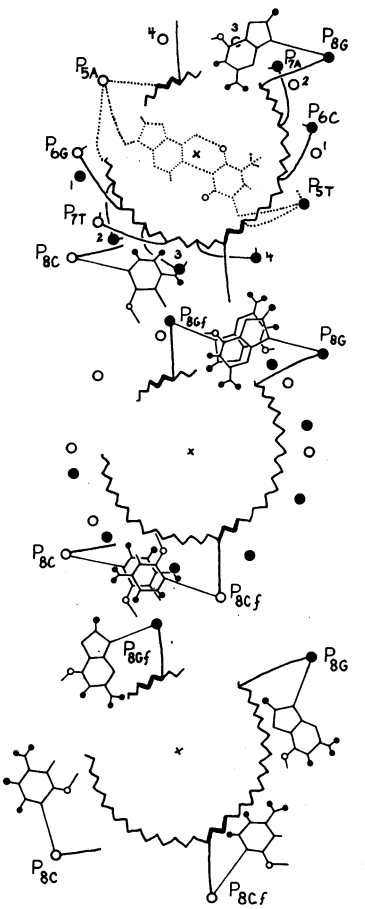

FIG. 3.—Alternative semiconservative replication scheme. In the previous scheme (Fig. 2) one strand gained a new partner while the other strand did peel off. In this scheme, both strands replicate in a semiconservative fashion. An ionic condition change causes P_{8G} to break from the wide groove protein side chain and P_{8C} to break from the narrow groove protein. Attached to one of the proteins at P_{8G}, the guanine 8G swings out as does also the cytosine 8C while attached to the other protein at P_{8C}. (cf. top of figure). A filial guanine 8Gf is held to the guanine 8G, and a filial cytosine 8Cf is held to the cytosine 8C, both by London forces (middle of figure), until attachments to the side chains of groove proteins are made at P_{8Gf} and P_{Cf}, respectively. The attachments lead to two double-stranded nucleic acids (bottom of figure), with each double helix attached to a corresponding protein chain. Here it must be specially emphasized that the three-dimensional structure is not conserved as the figures might suggest. The looseness of the structural conditions makes this replication scheme not quite as attractive as the previous one.

ionic conditions are re-established, complementary hydrogen bond base pairing of those new filial nucleotides will occur with the opposite single-stranded parental nucleic acid chain (which is, of course, complementary to the filial chain). This process amounts to a "semiconservative" scheme of replication with a single strand functioning as a half of the "primitive duplex." [11] The phosphodiester linkages along the filial strand may then be formed, the energy being provided from the activation of the filial nucleotides.

Alternative Scheme.[12]—Figure 3 indicates a variation of the scheme in which both halves of the parental double-stranded helix enter into making two new semiconservative double-stranded helixes. This scheme differs from that shown in Figure 2 in that now two amino acid attachments are broken and that the prying-open process brings nucleotides from both parental chains into a position of exposure of their bases (upper part, Fig. 3). Both strands of the parental helix collect by London forces "filial" nucleotides identical to the parental ones, and the "filial" phosphates may attach to the two free amino acid side chains (middle part, Fig. 3). Now they may readily base-pair (lower part, Fig. 3). It is to be noted that the conserved nucleic acid single strands are now each attached to one of the protein chains which makes their orderly separation possible. Both nucleic acid strands replicate in a semiconservative manner. We prefer, however, the scheme of Figure 2 because it is structurally more specific and because it does not require simultaneous synthesis at both C_3' and C_5' positions.

The scheme of Figure 3 was designed to represent a Y-formed replication process, both arms replicating. It is to be noted that such a process may actually consist of a sequence of two processes, each of the type represented by Figure 2, one lagging behind the other; in both procedures it is one and the same strand which functions as the immutable parental strand. Cairn's and Meselson-Stahl's experiments may thereby be interpreted.

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We are deeply indebted to Miss Dorothy Aein for discussions and suggestions.

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duplex") rather than the two strands of a single Watson-Crick helix (a "primitive duplex"). L. F. Cavalieri's work pointed to the interesting possibility of such an occurrence (*Biophys. J.*, **1**, 317 (1961). C. Shooter, R. Baldwin, and R. Inman, however, give data showing strong evidence to the effect that a primitive duplex underlies the Meselson-Stahl experiments.

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*REPLICATIVE FRAGMENTATION IN T4 PHAGE: INHIBITION BY CHLORAMPHENICOL**†

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Reproduction of the T-even phage, known to undergo several rounds of mating in the vegetative pool inside the infected bacterial host, is an example of a semiconservative type of DNA replication which is paralleled by its fragmentation and rejoining.[1, 2] This is in contrast to a copy choice mechanism which would not lead to fragmentation of parental DNA. The parental label contribution to a single progeny phage is no more than 7 per cent, incorporated into the progeny molecule as a semiconservative fragment (or fragments). It has been proved that the parental fragment, incorporated into the progeny polynucleotide strand, is integrated, i.e., that there are no breaks in the polynucleotide chain at either end of the parental segment.[3] The system involved in the exchanges during mating provides a mechanism for repairing the integrity of the polynucleotide chain. Whether these exchanges are the exclusive affair of DNA molecules or whether a specific enzyme is involved was unknown.

This paper will demonstrate that fragmentation of the parental phage DNA, as described above, can be inhibited by chloramphenicol (CM) which, however, does not affect semiconservative replication. A catalyst, presumably an enzyme, is required for fragmentation.

Materials and Methods.—(*a*) The bacterial strain used in these experiments was *E. coli* B. The bacteriophage used was an osmotic shock resistant mutant $T_4BO_1^r$. Synthetic TCG medium (light, heavy, and radioactive), along with methods of purification, assays of bacteriophage, techniques of CsCl density gradient centrifugation, and methods for fraction collection have been previously described.[1, 3]

(*b*) The net synthesis of DNA was measured as the uptake of P^{32} into the alkali-resistant fraction obtained after fractionation of infected bacteria by the Schmidt-Thannhauser procedure.[4]

(*c*) DNA extraction from bacteriophage was performed as described before.[3] The intracellular phage DNA was extracted as follows: samples of infected bacteria,