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Biosynthesis of Nucleic Acids

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INTEREST in the biosynthesis of nucleic acids stems from the crucial importance of these compounds in heredity and reproduction and in the development of the cellular machinery.

Every cell has DNA in its nucleus and, from a variety of considerations, DNA is regarded as the prime source of genetic information. Consider a cell which just has been pinched from its mother and which has little more than a faithful replica of the maternal DNA. In its growth to maturity, the young cell will have to make the enzymatic machinery to carry on energy metabolism on an expanding scale. At the same time, the cell must synthesize all of the special structures (be they lipid, carbohydrate, protein, or of any other special nature) that will characterize the shape, structure, appearance, and behavior of the cell. The sequence can be represented, oversimplified, as

$$\text{DNA} \rightarrow \text{enzymes} \rightarrow \text{everything else.}$$

From all indications, it can be said with some confidence that the structure of DNA is fixed, that it does not undergo turnover under the most extreme variations of cellular nutrition and physiology, and, facetiously, that it is immutable, except for mutations. Aside from having to serve as an original template for the synthesis of the enzymes, it has one other major function and that is to provide the template for the synthesis of a replica of itself in the reproductive act of forming a daughter cell. Discussion of the biosynthesis of DNA is limited here to its replication during reproduction.

RNA BIOSYNTHESIS

What is known about the function and biosynthesis of RNA? In contrast with DNA, this problem appears more complex. RNA occurs both in the nucleus and in a variety of cytoplasmic structures. Its concentration in the cell may vary within wide limits depending upon the age, nutrition, and other environmental factors, and it obviously undergoes considerable turnover and replacement. While the DNA of the chromosome might be regarded as a single, exceedingly complex molecular unit, RNA is, by comparison, physically and metabolically heterogeneous and there is no reason to speak of RNA in the singular, except in the generic sense that one regards protein.

It would appear from current studies that RNA may provide a link between DNA and protein synthesis, translating the information in the DNA code to a form that is a proper template for the manufacture of distinctive proteins. What is known, in fact, from the work of Zamecnik *et al.*[1] with animal cells, is that RNA is essential for the assembly of amino acids into polypeptides, and from the work of Berg[2] with a bacterial system, is that only about 5 to 10% of the RNA seems to be active in the fixation of amino acids. This function of RNA as a link between DNA and enzyme synthesis is at present an educated and attractive speculation only.

Regarding the biosynthesis of RNA, the problem is complicated by its heterogeneity and by ignorance of its functions. There is a large and rapidly growing literature on the subject, but brief reference is made to only a few of the contributions.

(1) Ochoa[3] isolated from a variety of bacterial cells an enzyme which condenses various nucleoside diphosphates to high molecular-weight ribonucleic-acid-like polymers. These, discussed by Doty (p. 107) and by Rich (p. 191), are polymers of adenylic, cytidylic, or uridylic acid, or of other nucleotides or mixtures of them. As seen in Fig. 1, the reaction involves a condensation between the hydroxyl group in position 3 of one nucleotide with the inner phosphate group of the other nucleotide, with a consequent elimination of inorganic orthophosphate (Pi). There is little free-energy change in this reaction and, as indicated, the polymers are split by inorganic phosphate at physiological levels. For this enzyme to form the highly specific polymers required in protein synthesis, it must have controls in the cell from which it has escaped upon isolation. A reasonable possibility is that this enzyme provides for the conservation and storage of nucleotide units which can be called upon to form the adenylic, uridylic, cytidylic, and other coenzymes essential for energy metabolism, for carbohydrate, protein, and lipid biosynthesis, and for specific types of RNA.

(2) Several groups of investigators[4–9] have recognized, during the past year or so, the existence of enzyme systems which extend ribonucleic-acid chains by one or a few nucleotide units, and which employ nucleoside triphosphates rather than the diphosphates. There are suggestive indications of relationships of these reactions to amino-acid fixation leading to polypeptide formation.

(3) A reaction which has the intriguing possibility of relating RNA synthesis to DNA is the one recently described by Hurwitz.[10] An enzyme from *E. coli* was found which required DNA for the incorporation of ribonucleotides into a polymeric structure. With a mixture of radioactive ribonucleoside triphosphates and deoxynucleoside triphosphates as substrates, the prod-

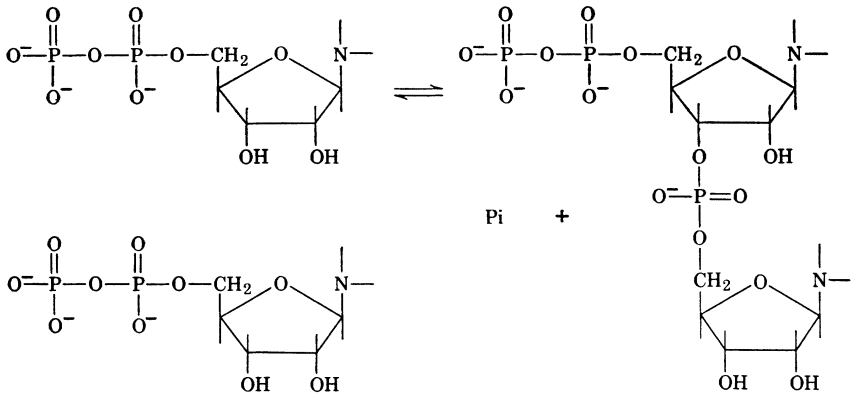


FIG. 1. Mechanism of RNA synthesis from ribonucleoside diphosphates.

uct formed was shown to contain the incorporated units in typical 3′-5′-phosphodiester linkage. Samples of RNA from several sources failed to replace the DNA requirement. Further purification of this enzyme system should clarify the specificities of the reaction and they are awaited with great interest.

(4) Turning now to studies with whole cells which attempt to relate nucleic-acid and protein synthesis, mention should be made first that, by the addition of chloromycetin to growing bacterial cultures, it is possible to suppress protein synthesis completely and still maintain the new formation of RNA and DNA. The synthesis of RNA, however, can be shown to be dependent upon the availability of all of the amino acids essential for protein synthesis.[11–13] Thus, a strain of *E. coli* requiring tryptophan for growth fails to make RNA in the presence of chloromycetin unless tryptophan is added to the medium. Yet this and other amino acids for which this effect has been demonstrated do not appear to be precursors of RNA. A dependence of RNA synthesis upon amino acids, despite the exclusion of protein formation, is implied, therefore, but an enzymatic basis for these observations as yet is not apparent.

(5) Finally, the studies of RNA biosynthesis in virus-infected bacterial cells are noteworthy. In a logarithmically growing population of bacterial cells, there is an exponential increase of protein, RNA, and DNA. At the time of virus infection, as with *T2* infection of *E. coli*, synthesis of bacterial components ceases abruptly and the cellular machinery is devoted completely to the synthesis of viral protein and viral DNA. Since the virus lacks RNA, there is—or rather, there was thought to be—no RNA synthesis. Recent tracer studies by Volkin and Astrachan[14] have shown a burst of RNA synthesis, very small in amount but distinctive in composition. One interpretation of these findings is that this RNA synthesis is in response to the unique instructions carried in the viral DNA, and is a necessary prerequisite for the formation of the proteins essential to virus formation. There is here a highly simplified situation in which to study a form of RNA biosynthesis that is relevant to the link between DNA and protein and that is inviting to biochemical study.

DNA BIOSYNTHESIS

To review briefly the chemical structures which serve as the basic building units of the DNA molecule (Fig. 2): Deoxyadenylate (deoxyadenosine 5′-phosphate) is composed of a purine base linked by a glycosidic bond to the sugar that is unique to DNA, 2-deoxyribose. It is in the form of a furanose ring and lacks an oxygen at the 2-carbon position; esterified at carbon-5 is a phosphate residue. Thymidine 5′-phosphate, or thymidylate, is composed of a pyrimidine, thymine, linked as in the case of adenine to a 2-deoxyribose 5′-phosphate. The methyl group on the 5 carbon distinguishes thymine from uracil, also a pyrimidine, which is a component of

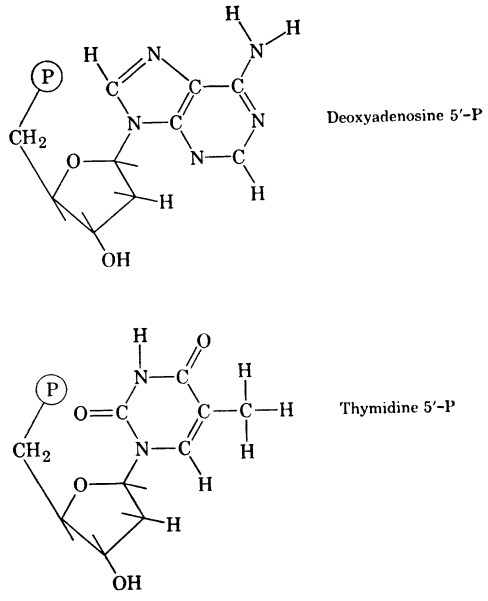


FIG. 2. Structures of a purine and a pyrimidine deoxynucleotide.

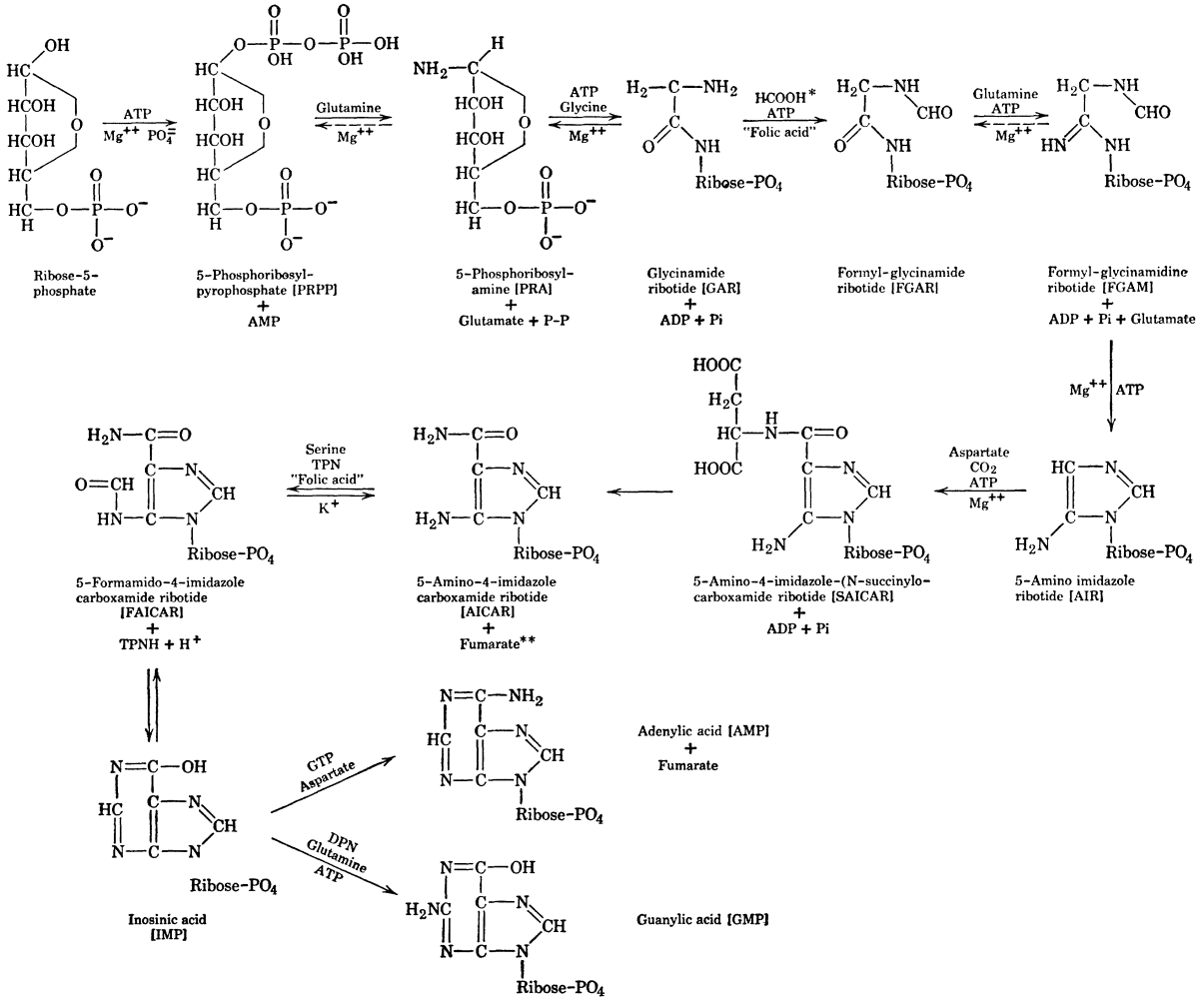


FIG. 3. Schema of enzymatic synthesis of purine ribonucleotides [from J. M. Buchanan, J. G. Flaks, S. C. Hartman, B. Levenberg, L. N. Lukens, and L. Warren, *Chemistry and Biology of Purines*, Ciba Foundation Symposium (J. and A. Churchill, Ltd., London, 1957), p. 233].

ribonucleic acid and of coenzymes prominent in carbohydrate metabolism. These and two other deoxynucleotides—a purine one, deoxyguanylate, and a pyrimidine one, deoxycytidylate—comprise the four deoxynucleotides which commonly occur in samples of DNA from bacterial, plant, and animal cells, and from some of the viruses. How are these units assembled?

This type of biosynthetic question has been approached in several ways. Earlier, Calvin (p. 147) and Roberts (p. 170) described the use of isotopic tracers to chart biosynthetic pathways. A suspected intermediate containing an isotopic marker is administered in the medium, and the cellular constituents which incorporate this marker are identified after brief or extended time exposures. A variation of this technique is to label the exclusive carbon or nitrogen source of the cell (e.g., C^{14} glucose or N^{15} ammonia), and then to determine whether or not certain (unlabeled) compounds in the medium can reduce the specific radioactivity of particular molecules in the cell under study.

Quite another approach involves the use of a mutant which accumulates intermediates because of an enzymatic deficiency at some point in the biosynthetic process. The facility with which mutants can be selected for service in a particular situation has made this technique extremely versatile and effective. These and other quite diverse methods have been used with considerable success in mapping pathways in the intact cell. But no combination of these methods can establish unequivocally a biosynthetic sequence! The walls and membranes of the cell form an "iron curtain" which either prevents certain molecules from entering the cell or alters them upon their entry or exit. It is a curtain which obscures the details to the point where a spur from a pathway is indistinguishable from the main line. Hence, a study of the enzymatic apparatus of the disrupted cell is an indispensable approach to the problem of biosynthesis. The objective of such an enzymatic attack on the problem is the isolation from cells of separate enzymes, each of which effects a single,

chemically rational step. These steps arranged in proper sequence should lead to the total synthesis of complex molecules. To assume real significance, the rates and conditions under which a reconstructed biosynthetic pathway operates ultimately must be reconciled with all of the isotopic, nutritional, and other observations made with the intact cells.

Turning now to what is known about the details of the enzymatic synthesis of the nucleotides (Fig. 3), one sees an outline of the discrete steps in the synthesis of adenosine 5′-phosphate starting with ribose 5-phosphate, glycine, glutamine, and, as an energy source, adenosine triphosphate (ATP). This knowledge is largely the result of the work of Buchanan[15] and of Greenberg[16] and their colleagues. A comparable scheme could be shown for the pyrimidine nucleotides. The origin of the deoxyribonucleotides would appear, from current indications, to derive from the ribonucleotides by a direct reduction step.

Several years ago, experimental work was begun by our laboratory to determine the biochemical mechanism for the replication of DNA chains. One might suggest many permutations for how such chains might be

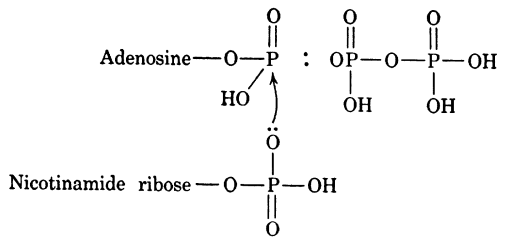


FIG. 4. Nucleophilic attack of nicotinamide mononucleotide on ATP in DPN synthesis.

assembled. We were guided by the knowledge of how the simplest and best known of the complex nucleotides, the coenzymes, are synthesized by the cell.[17–19] For example, the enzymatic synthesis of diphosphopyridine nucleotide (DPN) (Fig. 4) involves a condensation of ATP with nicotinamide mononucleotide and a resultant elimination of inorganic pyrophosphate (PP). Similarly, in the synthesis of flavin adenine dinucleotide (FAD), or coenzyme-*A*, there is a reaction of flavin mononucleotide or pantetheine phosphate, respectively, with ATP, and again inorganic pyrophosphate is eliminated (Fig. 5).[20] In each case, the adenyl coenzyme is produced by a reaction with an activated adenyl derivative such as ATP. More recently, the enzymatic synthesis of the adenyl derivatives of the fatty acids and amino acids have been shown to proceed by a similar mechanism.[21] What is shown in Fig. 5 to apply to the synthesis of adenyl derivatives applies also to the synthesis of uridyl, cytidyl, and guanosyl coenzymes.[22–24] Along lines proposed by Koshland,[25] one may visualize (Fig. 4) a nucleophilic attack on the activated adenyl derivative, in the case of DPN synthesis, by nicotinamide mononucleotide, to form an anhydride bridge with the adenyl group and to displace inorganic pyrophosphate. By analogy, it can be conjectured that chains of nucleotides might be formed by a reaction of the end of a chain with an activated nucleotidyl molecule, such as a nucleoside triphosphate. In other words, it is assumed that the basic building block is a 5′-phosphate ester of a deoxynucleoside, activated perhaps by linkage to a pyrophosphate group. As shown in Fig. 6, the end of a DNA chain might be

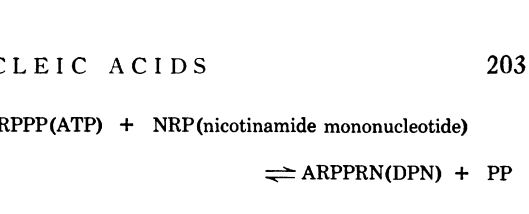


FIG. 5. Synthesis of adenine-nucleotide coenzymes.

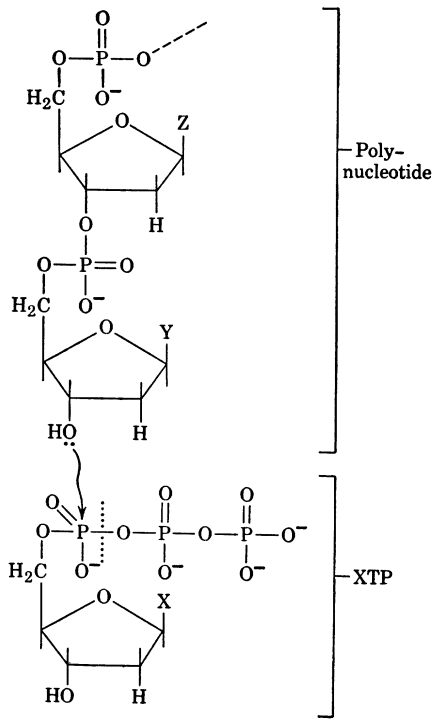


FIG. 6. Postulated mechanism for extending a DNA chain.

TABLE I. Enzyme purification.

Fraction number	Step	Units[a] per ml	Units[a] Total	Protein mg per ml	Specific activity units per mg protein
I	Sonic extract	2.0	16 800	20.0	0.1
II	Streptomycin	13.0	19 500	3.0	4.3
III	DNase, dialysis	12.1	18 100	1.80	6.7
IV	Alumina gel	15.4	12 300	0.78	20.
V	Concentration of gel eluate	110.	9 000	4.90	22.
VI	Ammonium sulfate	670.	6 030	8.40	80.
VII	DEAE resin	120.	3 600	0.60	200.–400.

[a] A unit is defined as the amount of enzyme causing the incorporation of 10 mμmoles of thymidine triphosphate into an acid-insoluble fraction during the assay period of 30 min at 37°. See Table II for further details about the assay incubation mixture.

involved in a nucleophilic attack on a deoxynucleoside triphosphate, thus extending the chain by one unit and displacing pyrophosphate.

We approached the problem of nucleic-acid synthesis experimentally by mixing together and incubating the following: a deoxynucleoside labeled very intensely in one of the carbon atoms of its base, an extract made from an exponentially growing culture of *E. coli* (generation time 20 min), ATP as an energy source, and Mg ions. To determine whether any nucleic-acid synthesis had taken place, one relied upon the very simple and fortunate chemical property of DNA that it is precipitated at acid *p*H, while the deoxynucleotides which were the substrates are completely soluble under these conditions. It was ascertained many times that thorough washing of the DNA precipitate removed even the smallest traces of the deoxynucleoside substrates. Thus, in our earliest experiments, traces of radioactive thymidine (thymine deoxyriboside) were found incorporated into an acid-precipitable substance that could be rendered *non*precipitable by treatment with purified deoxyribonuclease (DNase).[18] In this crude system, it was possible to detect 50 counts in the precipitate out of 5 000 000 counts in 1 μmole of the starting substrate. This indicated an incorporation of only 10 μμmoles or about 1/10 000 of the amount detectable by the most sensitive microchemical methods.

Using the incorporation of C^{14}-thymidine into a DNase-sensitive acid-precipitable material as the assay, purification was begun of the components of the *E. coli* extract essential for this reaction. It soon was recognized that one of the enzymatic functions of the crude fraction was the conversion of the thymidine through thymidylate to the triphosphate level, and thymidine triphosphate was used thereafter as the substrate.[26,27]

The enzyme-purification procedure at present[28] involves first the preparation of an extract of *E. coli* by sonic treatment, then several fractionation steps (Table I) resulting in an enrichment with respect to protein of about 2000 to 4000 times over the crude extract. With this preparation, certain interesting features of the reaction have become apparent and are discussed presently.

Even at an intermediate stage of purification, it became clear that, in order for one of the triphosphates to be incorporated into DNA, the triphosphates of all four of the deoxynucleosides which commonly occur in DNA, and also DNA itself, must be present.[29] This is illustrated in Table II. The incubation mixture contained the triphosphates* of thymidine, deoxycytidine, deoxyguanosine, and deoxyadenosine, calf-thymus DNA, Mg^{++}, and enzyme (with the most purified of the enzyme preparations, about 0.1 μg of protein was used). The isotopic marker used has been either C^{14} in carbon-2 of thymine or P^{32} in the innermost phosphate group of one of the triphosphates. Under these conditions, 0.5 mμmole of the labeled nucleotide was incorporated. Omission of any one of the triphosphates, of the DNA, or of Mg^{++}, or pretreatment of the DNA with crystalline pancreatic DNase reduced the reaction to a level below the limits of detectability. Replacement of the triphosphates by the corresponding deoxynucleoside diphosphates reduced the reaction to or near the limits of detectability (<5%). Nor was there any detectable reaction when ribose analogs such as ATP or CTP were used in place of dATP or dCTP, respectively. Also, there was no reaction when DNA was replaced by RNA or by DNA degraded by acid treatment or sonic irradiation. However, as is mentioned later, DNA from a variety of sources, plant, animal, and virus, served in the reaction.

The enzymatic incorporation of deoxynucleotide into a DNA fraction using incubation conditions similar to those in Table II also has been observed with the use of sonic extracts of other bacterial species (*Hemophilus influenzae*, *Aerobacter aerogenes*), and with extracts of several types of animal cells (HeLa cell cultures, lymph-gland and leukemic cells).[29] While assays for the DNA-

TABLE II. Requirements for deoxynucleotide incorporation into DNA.

	mμmoles
Complete system[a]	0.50
Omit dCTP, dGTP, dATP	<0.01
Omit dCTP	<0.01
Omit dGTP	<0.01
Omit dATP	<0.01
Omit Mg^{++}	<0.01
Omit DNA	<0.01
DNA pretreated with DNase	<0.01

[a] The complete system contained 5 mμmoles of TP^{32} PP (1.5×10^6 cpm per μmole), dATP, dCTP, and dGTP, 1 μmole of $MgCl_2$, 20 μmoles of glycine buffer (pH 9.2), 10 γ of calf-thymus DNA, and 3 γ of enzyme Fraction V, in a final volume of 0.30 ml. The incubation was carried out at 37° for 30 min.

DNase treatment of DNA was carried out in an incubation mixture containing 60 γ of thymus DNA, 50 μmoles of Tris buffer, *p*H 7.5, 5 μmoles of $MgCl_2$, and 5 γ of pancreatic DNase, in a final volume of 0.50 ml. After 30 min at 37°, 0.02 ml of 5% bovine serum albumin and 0.05 ml of 5*N* perchloric acid were added. The precipitate was centrifuged and dissolved in dilute alkali and neutralized. A control incubation from which DNase was omitted yielded fully active DNA.

* For the triphosphates of thymidine, deoxycytidine, deoxyguanosine, and deoxyadenosine, respectively, the following abbreviations are used: TTP, dCTP, dGTP, and dATP. The symbol $TP^{32}PP$ indicates that the triphosphate is labeled with P^{32} in the innermost phosphate group.

synthesizing system cannot be regarded as accurate in the crude extracts, it is significant that the values for the bacterial extracts are of the order of 20 to 50 times greater than those for the animal-cell extracts. Bollum and Potter[31,32] have reported the incorporation of H^3-thymidine into DNA by extracts of regenerating rat liver.

With the most purified *E. coli* enzyme preparation, it has become possible to demonstrate net synthesis by the use of more-direct chemical methods. Several experiments are illustrated in Table III. In experiment 1, there was an increase in DNA of a little over twofold, measured by spectrophotometry, deoxypentose assay, or by incorporation of radioactive tracer. In the other experiments, increases of DNA by a factor of 10 to 20 were obtained, and 90 to 95%, therefore, of isolated DNA was derived from the deoxynucleotide substrates supplied. The factors responsible for cessation of the reaction are currently under study.

On the basis of the foregoing results, one can consider the following over-all equation for the enzymatic synthesis of DNA:

$$\left.\begin{matrix} n\ \mathrm{TPPP} \\ n\mathrm{dGPPP} \\ n\mathrm{dAPPP} \\ n\mathrm{dCPPP} \end{matrix}\right\} + \mathrm{DNA} \rightleftharpoons \mathrm{DNA} - \begin{bmatrix} \mathrm{TP} \\ \mathrm{dGP} \\ \mathrm{dAP} \\ \mathrm{dCP} \end{bmatrix}_n + 4(n)\mathrm{PP}$$

The four triphosphates+DNA yield a product which contains the four nucleotidyl residues linked in some covalent and hydrogen-bonded fashion with the DNA. It is apparent from the equation that inorganic pyrophosphate (PP) must be split out during the reaction and in quantities equimolar to the amounts of deoxynucleotide incorporated into the DNA. It might be considered also, especially in the light of the Ochoa studies[33] of the reversibility of synthesis of ribonucleic-acid polymers, that there might be a pyrophosphorolytic reversal of the reaction. It has been shown[18] that, with the incorporation of C^{14}-thymidylate into DNA, equimolar amounts of PP were released; this was isolated and identified by ion-exchange chromatography.

TABLE III. Net synthesis of DNA.

Expt. No.[a]	Estimation	Control (no enzyme)	Complete	Δ
		μmoles	μmoles	μmoles
1	P^{32} incorporation	0.00	0.28	0.28
	Optical density	0.19	0.46	0.27
	Deoxypentose	0.19	0.40	0.21
2	Optical density	0.06	0.63	0.57
3	Optical density	0.05	0.58	0.53
4	Optical density	0.05	0.64	0.59
5	Optical density	0.04	0.89	0.85

[a] In experiment 1, the incubation mixture (3.0 ml) contained 0.15 μmole of $dAP^{32}PP$ (1.3×10^6 cpm per μmole), 0.3 μmole of dGTP, 0.15 μmole of dCTP, 0.15 μmole of dTTP, 200 μmoles of potassium-phosphate buffer (*p*H 7.4), 20 μmoles of $MgCl_2$, 0.1 mg of calf-thymus DNA, and 12 γ of enzyme Fraction VII. The mixture was incubated at 37° for 180 min. DNA was precipitated, washed, taken up in 1.2 ml of 0.5*N* perchloric acid, and heated for 15 min in a boiling water bath. Optical-density measurements were made at 260 mμ and converted to nucleotide equivalents using a molar extinction coefficient of 8960 (derived from the calculated values for an acid hydrolyzate of calf-thymus DNA). In the P^{32} estimation of DNA synthesis, incorporation of deoxyadenylate was multiplied by a factor based on its percentage in calf-thymus DNA. The radioactivity actually observed for the controls did not exceed the background count. In experiments 2, 3, 4, and 5, the reaction mixture (1.0 ml) contained 0.32 μmole of each of the four triphosphates, 30 γ of calf-thymus DNA, 60 μmoles of potassium-phosphate buffer (*p*H 7.4), 6 μmoles of $MgCl_2$, and 8 γ of enzyme Fraction VII. The mixture was incubated for 250 min at 37°. 2*M* NaCl then was added to give a final concentration of 0.2*M* and the mixture was heated for 5 min at 70°. Unreacted triphosphates were removed by exhaustive dialysis against 0.2*M* NaCl. The product contained no acid-soluble material. Optical density at 260 mμ was determined and converted to nucleotide equivalents using a molar extinction for DNA of 6900.

TABLE IV. Physical properties of DNA.

	Primer	Product	Heated at 100°C, 15 min Primer	Heated at 100°C, 15 min Product
Sedimentation coefficient	25	20–25	20	14
Intrinsic viscosity	50	15–35	<1	<1
Molecular weight	8×10^6	$4\text{–}6\times10^6$	$<1\times10^6$	$<0.7\times10^6$

Evidence for reversal of the reaction has been obtained.[29] When PP is present in concentrations of 2×10^{-3} M—i.e., about 100 times the concentration of the deoxynucleoside triphosphates—the synthetic reaction is inhibited by about 50%. Under such conditions, when PP^{32} is used, its incorporation into the terminal PP groups of the four triphosphates has been observed. The rate of the reaction is comparable to the synthetic rate. The reaction is absolutely dependent upon the presence of DNA. DNA degraded with DNase is inert. Inorganic orthophosphate fails to replace PP. A distinctive feature of the reaction is that triphosphates are required. When they are omitted, there is only a small reaction, which may be interpreted as a very limited pyrophosphorolysis of DNA. It is necessary to have only one triphosphate to augment the pyrophosphorolysis reaction considerably.

Up to this point, the discussion has been concerned with how a purified enzyme preparation can synthesize, in the presence of four deoxynucleoside triphosphates and of DNA, presumably acting as primer, a product that is insoluble at acid *p*H and which is nondialyzable and may be degraded to acid-soluble fragments by the action of pancreatic DNase. It has been established with more-sensitive techniques that even a single triphosphate may react, but to an extent so limited as to suggest the addition of one or very few residues to the ends of the DNA chains added as acceptor.[34]

A more detailed consideration is presented below of the physical and chemical nature of the enzymatic product, DNA, prepared under conditions where 90% or more of the polymerized material is newly synthesized.

In physicochemical studies, we have been able to show that the enzymatically synthesized product has essentially the same physical characteristics as DNA carefully prepared from calf thymus.[35] The sedimenta-

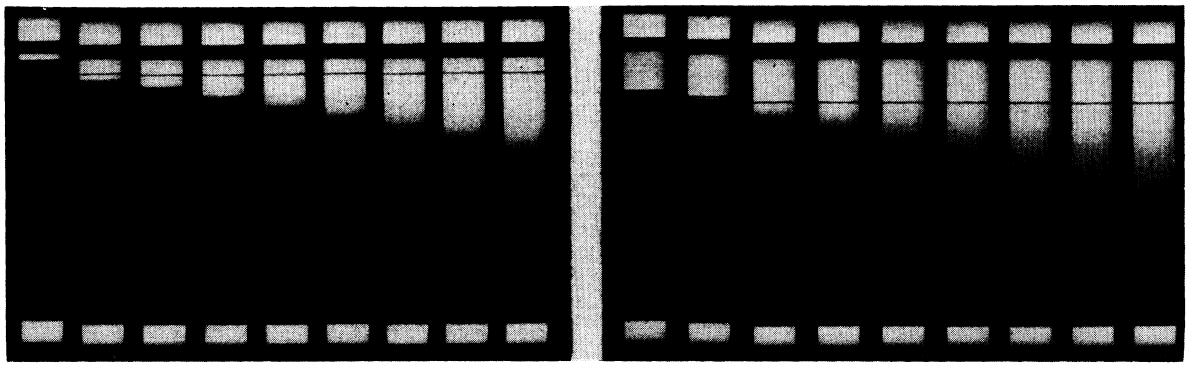


FIG. 7. Sedimentation of DNA primer and the enzymatically synthesized product.

tion behavior (Fig. 7) of the calf-thymus and enzymatically synthesized samples were quite similar, although the enzymatically synthesized sample showed a greater polydispersity. The latter may be the result of the action of contaminating DNases in the enzyme preparation. The sedimentation constants in this and in a great many other runs have been in the neighborhood of 25 to 30. Viscosimetric determinations also have yielded values of 15 to 35 deciliters/gram which are comparable to those obtained for calf-thymus DNA (40 to 50); from these, average molecular weights of 4 to 6 million for several samples of enzymatically synthesized DNA were calculated (Table IV). It may be inferred from the sedimentation and viscosity characteristics of the product that it consists of highly ordered rigid structures with effective volumes greater than would be expected from single polynucleotide chains with freedom of rotation at each link in the backbone. In support of this view was the collapse of the macromolecular structure when the DNA was heated for 15 min at 100°C; like thymus DNA, the viscosity decreased to immeasurably low levels, while the sedimentation rate decreased only slightly. Furthermore, there was found a typical hyperchromic effect[36] upon degradation of the product with DNase. The curves in Fig. 8 show that just as there is an increase in optical density upon digestion of calf-thymus DNA with pancreatic DNase, so is there a kinetically similar increase with the enzymatic product and to the same extent of about 30% above the starting values.

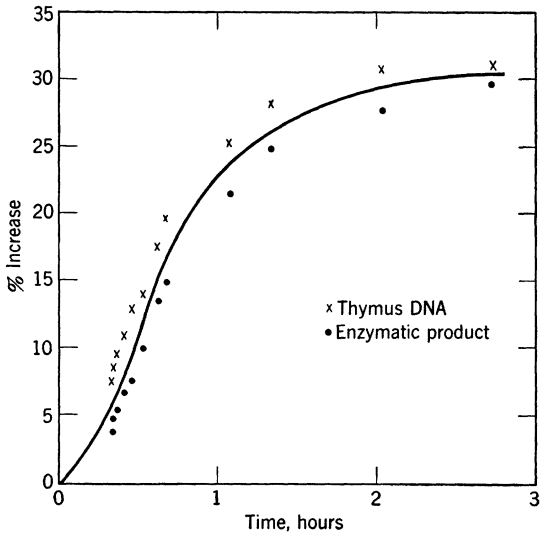


FIG. 8. Increase in ultraviolet absorption of DNA upon digestion with pancreatic DNase.

One now comes to a consideration of the chemical structure of the product. To begin with, it can be affirmed that the substrates are linked in the DNA product by typical 3′–5′ diester bonds.[29] What can be said of the base composition and the ratios of bases of the product? Do they bear any relation to the DNA primer added to the reaction?

In Table V are the base-composition data on enzymatically synthesized DNA. A close correspondence exists in the enzymatically synthesized DNA's between the contents of adenine and thymine on the one hand and of guanine and cytosine on the other, so that the ratio of purines to pyrimidines (A+G/C+T) is in each case nearly unity. Furthermore, good agreement exists for the ratio (A+T/G+C) between the enzymatic product and its corresponding primer, ranging from values of 0.59 for *Mycobacterium phlei* to greater than 40 for an enzymatically synthesized copolymer of deoxyadenylate and thymidylate. The latter is formed by the DNA-synthesizing enzyme in the absence of primer under rather specialized, but poorly understood, conditions: specifically, after lag periods of from 3 to 6 hr. Once formed, such a polymer then can be replicated without a lag and will consist solely of adenine and thymine, although all four of the deoxynucleoside triphosphates are provided in the reaction mixture. This polymer, therefore, represents an extreme case in which the base composition of the enzymatic product reflects that of the primer.

TABLE V. Purine and pyrimidine composition of enzymatically synthesized DNA.[a,b]

DNA		Number of analyses	A	T	G	C	$\frac{A+T}{G+C}$	$\frac{A+G}{T+C}$
M. phlei	primer	3	0.65	0.66	1.35	1.34	0.49(0.48–0.49)	1.01(0.98–1.04)
	product	3	0.66	0.80	1.17	1.34	0.59(0.57–0.63)	0.85(0.78–0.88)
A. aerogenes	primer	1	0.90	0.90	1.10	1.10	0.82	1.00
	product	3	1.02	1.00	0.97	1.01	1.03(0.96–1.13)	0.99(0.95–1.01)
E. coli	primer	2	1.00	0.97	0.98	1.05	0.97(0.96–0.99)	0.98(0.97–0.99)
	product	2	1.04	1.00	0.97	0.98	1.02(0.96–1.07)	1.01(0.96–1.06)
Calf thymus	primer	2	1.14	1.05	0.90	0.85	1.25(1.24–1.26)	1.05(1.03–1.08)
	product	6	1.19	1.19	0.81	0.83	1.46(1.22–1.67)	0.99(0.82–1.04)
T2 phage	primer	2	1.31	1.32	0.67	0.70	1.92(1.86–1.97)	0.98(0.95–1.01)
	product	2	1.33	1.29	0.69	0.70	1.90(1.82–1.98)	1.01(1.01–1.03)
"Synthetic A-T Copolymer"	···	1	1.99	1.93	<0.05	<0.05	>40.	1.05

[a] A, T, G, and C refer, respectively, to adenine, thymine, guanine, and cytosine, except that C in the case of *T2*-phage primer refers to hydroxymethylcytosine.
[b] The figures in parentheses represent the range of values obtained.

The somewhat higher values of (A+T/G+C) observed for some of the products most probably can be attributed to contamination of these DNA's with traces of this deoxyadenylate-thymidylate copolymer. The obvious implications of these early results are that the added DNA is serving as a template for an enzymatic replication of DNA, but it is evident too that more-extensive documentation is necessary before this conclusion can be considered to be established.

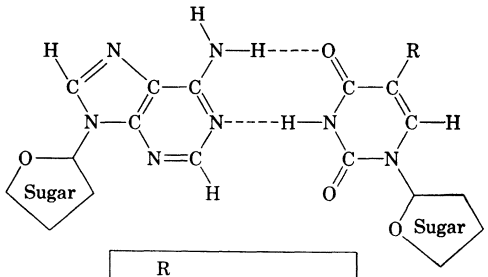
In reviewing the specificity of this DNA-synthesizing system, the results have indicated that samples of DNA from a variety of origins can serve as primers. It has been mentioned also that only the *tri*phosphates of the *deoxy*nucleosides are reactive. What can be said of the specificity of the substrates with respect to the structure of the pyrimidine and purine bases? From the many interesting reports on the incorporation of bromouracil,[37–39] of azaguanine,[40] and of other analogs into bacterial and viral DNA, it might be surmised that some latitude in the structures of the bases can be tolerated provided there is no interference with their hydrogen bonding. It would be well to reiterate at once what Rich mentioned earlier (p. 191). Analysis of the composition of samples of DNA from a great variety of sources and by many investigators (reviewed by Chargaff[41]) reveals the remarkable fact that the purine content always equals the pyrimidine content. Among the purines, the adenine content may differ considerably from the guanine, and among the pyrimidines, the thymine from the cytosine. There is an invariable equivalence, however, between bases with an amino group in the 6 position of the ring, such as in adenine and cytosine, and the bases with a keto group in the 6 position of the ring, such as in guanine and thymine. These facts[41,42] were interpreted on the basis of hydrogen bonding by Watson and Crick[43] in their masterful hypothesis on the structure of DNA. In a given species, the DNA composition of all of the cells is characterized by a distinctive ratio of the number of adenine-thymine pairs to the number of guanine-cytosine pairs.

TABLE VI. Replacement of natural bases by analogs in enzymatic synthesis of DNA.

Expt. No.	Control value[a] (mμmoles)	Base analog used	Natural base omitted (Percent of control)[b]			
			Thymine	Adenine	Guanine	Cytosine
1	0.50	Uracil	*54*	4	6	
1a	0.88	Uracil				3
2	0.43	5-bromouracil	*97*	2	4	
2a	0.42	5-bromouracil				4
3	0.51	5-bromocytosine		4	4	*118*
3a	0.40	5-bromocytosine	4			
4	0.58	5-methylcytosine		2	3	*185*
4a	0.52	5-methylcytosine	2			
5	0.37	Hypoxanthine		3	*25*	5
5a	0.27	Hypoxanthine	4			

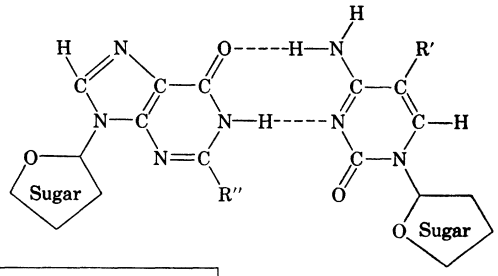
[a] Control values are mμmoles of radioactive deoxynucleotide incorporated into DNA in the absence of analog. Incubation mixtures contained in 0.3 ml. 5 mμmoles each of TTP, dATP, dCTP, and dGTP; 2 μmoles of $MgCl_2$; 20 μmoles of potassium phosphate (*p*H 7.4); 10 μg of calf-thymus DNA; and 1μg of enzyme fraction VII-R. Experiments were performed at 37° for 30 min. Labeled substrates were: $dCP^{32}PP$ in Expts. 1, 2, 5a; $TP^{32}PP$ in Expts. 1a, 3,4,5; and $dGP^{32}PP$ in Expts. 2a, 3a, 4a.
[b] The percentage value represents the fraction of the labeled substrate incorporated when the analog (5 mμmoles) was used instead of a natural base. All bases, natural or analog, were supplied as the deoxynucleoside triphosphates. Values of 5% or below are near the limit of detectability and are of questionable significance.

Hydrogen Bonding of Adenine to Thymine



R	
—CH_3	Thymine
—H	Uracil
—Br	Bromuracil

Hydrogen Bonding of Guanine to Cytosine



R′	
—H	Cytosine
—CH_3	Methylcytosine
—Br	Bromcytosine

R″	
—NH_2	Guanine
—H	Hypoxanthine

FIG. 9. Hydrogen bonding of the bases.

As can be seen from Table VI,[44] deoxyuridine triphosphate used in place of thymidine triphosphate supported DNA synthesis at 54% of the rate of the control value but failed to support synthesis when used in place of the triphosphates of deoxyadenosine, deoxyguanosine, or deoxycytidine. 5-bromodeoxyuridine triphosphate was more effective as a replacement for thymidine triphosphate but was unable to substitute for any of the other triphosphates. The 5-bromo- and 5-methyl-deoxycytidine triphosphates replaced only deoxycytidine triphosphate and, for unexplained reasons, were even more effective than deoxycytidine triphosphate itself. Deoxyinosine triphosphate permitted a reduced rate of DNA synthesis in the absence of deoxyguanosine triphosphate, but essentially no synthesis when any one of the other triphosphates was absent.

Thus, uracil and 5-bromouracil specifically replaced thymine; 5-methyl- and 5-bromo-cytosine replaced cytosine, and hypoxanthine substituted for guanine; xanthine was not incorporated into DNA. As seen from Fig. 9, the specific replacement of the natural bases by these analogs is consistent with and offers additional support for the base-pairing relationships in the double helix proposed by Watson and Crick for the structure of DNA.

The current status of knowledge of the biochemical aspects of RNA and DNA synthesis has been sketched in the foregoing. With respect to RNA, the enzymatic information available is inadequate to explain the metabolic behavior of cells and tissues. In the case of DNA, the enzymatic studies of replication can be reconciled with genetic phenomena, but much remains to be clarified in the mechanism of the reaction. While the biological implications of these problems are exciting and pressing, the most immediate obstacles are the limited resources available to separate and characterize these macromolecules. New techniques are desperately needed now in the nucleic-acid field to cope with such problems as were solved in the protein field over the last 50 years.

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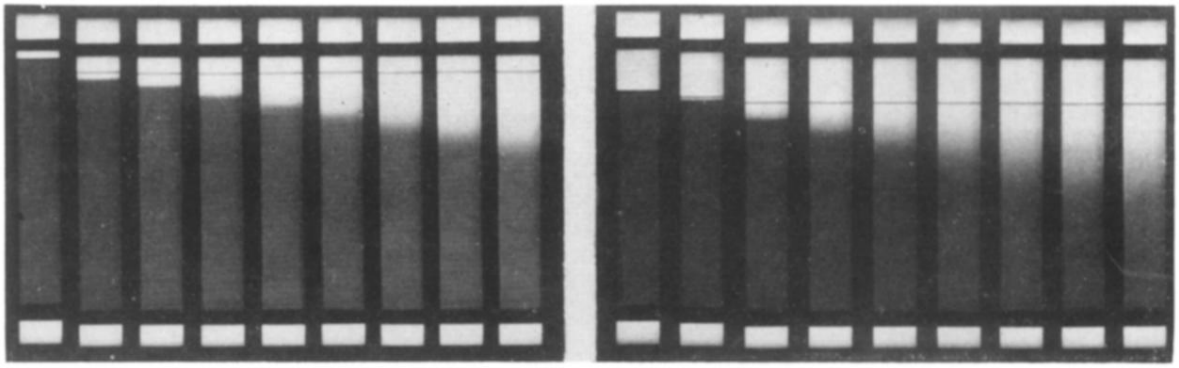

Calf-thymus DNA
$S = 24$

Enzymatically synthesized DNA
$S = 24$

FIG. 7. Sedimentation of DNA primer and the enzymatically synthesized product.