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CHEMICAL METHODS OF SYNTHESIS OF POLYNUCLEOTIDES

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I. INTRODUCTION

The problems of the synthesis of polynucleotides are now attracting attention from scientists. Enzymes have been used to obtain deoxyribopolynucleotides [1] and ribopolynucleotides [2-5] of high molecular weight (from 30 000 to 2 000 000) and a random distribution of purine and pyrimidine bases along the polynucleotide chain, which have played an exceptionally important role in investigations which have been undertaken as the start of the study of the amino-acid code [6,7]. For several investigations there is a need for polynucleotides of definite composition and comparatively low molecular weight [8,9]. In studying the influence of the chain length of polyuridylic acid on the inclusion of phenylalanine in a cell-free yeast system it was found that the polyuridylic acid is active from the nonanucleotide onwards [10]. A sharp increase in the inclusion of phenylalanine was noted on going from U_8† to U_9 from U_{11} to U_{12} and from U_{14} to U_{16} (up to U_{22} studied), but for definite conclusions it was necessary to check the results on pure specimens of the polynucleotides.

† The nomenclature, abbreviations, and representation in diagrams corresponds to that generally accepted [11,12].

Preliminary experiments with hexauridylic acid having a terminal 3'-phosphate group showed that in a mixture with a high-polymer polyuridylic acid the hexanucleotide inhibits its activity by ~30% in relation to the synthesis of polyphenylalanine [13]. When hexauridylic acid is treated with dicyclohexylcarbodiimide (DCC) under conditions leading to the cyclisation of the 3'-monophosphate into the 2',3'-cyclic phosphate, inhibition is not observed. An analogous effect, but with oligouridylic acids having a terminal 5'-phosphomonoester group was observed by Jones and coworkers [14]. The dependence of the synthesis of peptides of lysine on the length of the polyadenylic acid chain has also been noted [15].

Homopolynucleotides with different terminal groups are frequently used in work to determine the specificity of nucleases [16-18] and as formerly is of interest for determining the sequence of the purine and pyrimidine bases in the nucleic acids and in the investigations of enzymes which synthesise the nucleic acids [19].

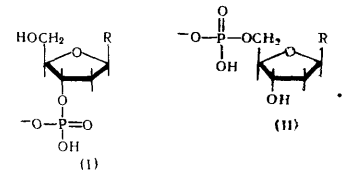
The availability of polynucleotide with a chain of known dimensions, known composition, and with known terminal groups should facilitate the study of systems which synthesise polypeptides, and apparently show the direction of further work on the synthesis of specific ribopolynucleotides of interest in the study of the amino-acid code [20]. The problems connected with obtaining such polynucleotides should also be solved by chemical methods of polynucleotide synthesis.

Chemical methods of synthesising polynucleotides are developing in two directions: (1) the stepwise synthesis of oligonucleotides, and (2) polycondensation. The stepwise synthesis of oligonucleotides recently formed the subject of a special review [21]. The present review will consider methods of synthesising polynucleotides by condensation‡ of mononucleotides or other "monomeric" units.

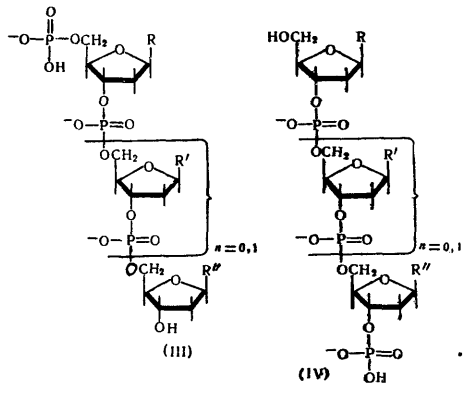
II. METHODS OF POLYMERISATION

1. Types of "Monomers"

It must be specially emphasised that in developing methods of synthesis of polynucleotides it must be taken into account that the polymeric chain should be formed by molecules of sugar (deoxy-D-ribose or D-ribose) joined by residues of phosphoric acid through the OH-group at the $C_{(3')}$ atom of one carbohydrate molecule and the OH group at the $C_{(5)}$ atom of another. In accordance with this requirement, the "monomers" for obtaining the polynucleotides can be derivatives of nucleotides of the following types: (a) for the deoxyribopolynucleotides series:

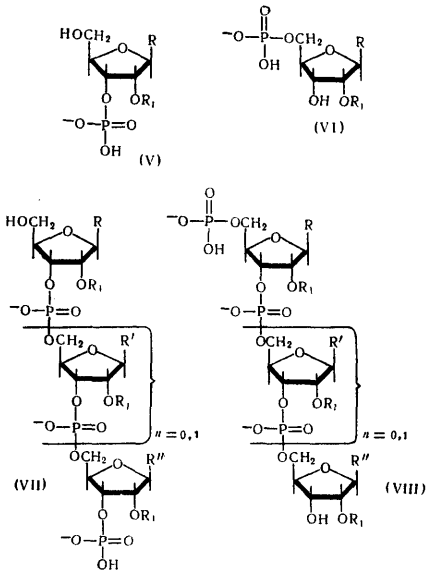


‡ Usually in the literature devoted to the problems of obtaining polynucleotides this method is inaccurately termed polymerisation; we will keep to this name subsequently as it is the most widely employed.



(III) (IV)

(b) for the ribopolynucleotides series:



(V) (VI) (VII) (VIII)

where R, R′, and R″ are purine or pyrimidine bases with protected amino-groups for adenine, guanine, and cytosine, and R_1 is acyl or alkyl. Of the types of monomer listed, the following have been used for the synthesis of polynucleotides:

(I) R = thymine [22-24].
(II) (a) R = thymine [25,26]; (b) R = *N*-benzoyladenine [27]; (c) R = *N*-anisylcytosine [28]; (d) R = *N*-acetylguanine [29].
(III) n = 0; (a) R = *N*-anisylcytosine, R″ = *N*-acetylguanine [12]; (b) R = thymine, R″ = *N*-benzoyladenine [30]; (c) R = *N*-anisylcytosine, R″ = thymine [31].
(V) (a) R = *N*-benzoyladenine, R_1 = acetyl [32]; (b) R = = uracil, R_1 = acetyl [13,33].

Khorana and coworkers [30] proposed to obtain a monomer of type (IV), but there has not yet been any work in this direction. Monomers of types (VI) and (VIII) cannot be obtained at present (there are still no methods which allow the hydroxyl group at the $C_{(2')}$ atom to be selectively protected when there is a free hydroxyl group at $C_{(3')}$). Monomers of type (VII) can be obtained but have not yet been described. There is only a brief reference [34] to the use of the following trinucleotides as a monomer: (1) (VII) with n = 1, R = adenine, R′ = guanine, R″ = cytosine, R_1 = = H; (2) (VII) with n = 1, R = adenine, R′ = guanine, R″ = = uracil, R_1 = H.

In addition to those listed, the starting materials for polymerisation have included nucleoside-2′,3′ cyclicphosphates [35], but monomers of this type form both $C_{(3')}$–$C_{(5')}$ and $C_{(2')}$–$C_{(5')}$ internucleotide bonds in polymerisation, therefore they are not of great interest (see below for details).

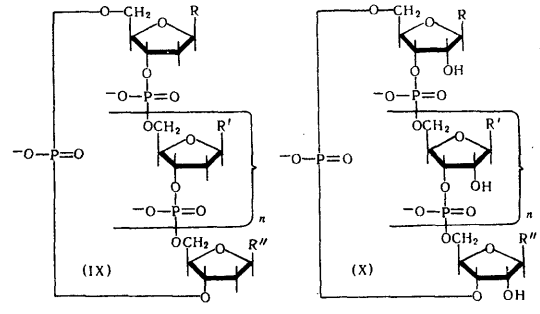
2. Conditions for the Polymerisation, Isolation, and Identification of Polynucleotides

The polymerisation of appropriate monomers is usually brought about by use of such activating agents as DCC [dicyclohexylcarbodi-imide (Ed. of Translation)][25], *p*-toluenesulphonyl chloride [25], diphenyl phosphorochloridate [36], tetraethyl pyrophosphate [34], enol phosphates [37], polyphosphates [38], etc. The analysis of mixtures obtained in polymerisation in the presence of the above substances shows that the most suitable activating agent is DCC, since with all the others the mixture is more complex. An attempt to obtain a more homogeneous reaction mixture by the carbodi-imide method, using di-isopropylcarbodi--imide, was not successful, since in the presence of the latter polynucleotides with a rather less degree of polymerisation are obtained [11].

Usually the polymerisation is carried out in anhydrous pyridine solution at room temperature for 4–7 days. The concentration of the monomer in the reaction mixture is 1 M, that of DCC up to 2.5 M. The mixture of polymers is usually subjected to dialysis and separated by chromatography on DEAE or ECTEOLA-cellulose and paper chromatography. The chain length is determined by enzymic splitting of the corresponding polynucleotide to the nucleoside-3′ phosphate and nucleoside with subsequent determination of their concentrations by the methods of paper chromatography and spectrophotometry, and also by direct comparison with specimens obtained by partial enzymic splitting of the corresponding polynucleotide of high-molecular weight [13].

It has been found that the reaction mixture is quite complex: it contains linear polynucleotides, cyclic polynucleotides, non-symmetrical pyrophosphates, nucleoside-2′,3′ or nucleoside-3′,5′ cyclic phosphates, 5′-*C*-pyridinium nucleoside-3′ phosphates, and also several unidentified side-products.

The linear polynucleotides — (III), n = 0–10; (VII), n = = 0–8 — contain a different number of nucleotide residues, depending on the initial monomer, but not more than 12 (linear polynucleotides of higher molecular weight are present in the reaction mixture but they were not identified):



(IX) (X)

where R, R′, and R″ are purine or pyrimidine bases.

The cyclic polynucleotides (IX) and (X) are formed in large amounts (up to 16–18%) (with increase in the chain length their proportion decreases) and for pyrimidine monomers cyclic dinucleotides — (IX), (X), $n = 0$ — are formed predominantly[25], whereas for purine monomers, the formation of cyclic trinucleotides — (IX), (X), $n = 1$ — is characteristic[27,29]. Mixed monomers — for example, (III)(b) — form cyclodinucleotides[12].

To suppress the formation of cyclic forms, attempts were made to find new conditions for polymerisation[26]. The derivative of another nucleotide which would be the start of polymeric chains incapable of cyclisation was added to the mixture being polymerised, more concentrated solutions were used, monomers were taken admixed with a derivative of the same nucleotide but having only one phosphate group free — for example; (1) pT + pT-OAc§ (3 : 1)[26]; (2) (d)-pC^{An} + (d)-C^{An}-OAc (3 : 1)[28]; etc. The use of such a mixture for polymerisation is especially desirable in the ribonucleotide series, since the nucleoside-3′ phosphates — monomers of type (V) — have a greater tendency to form intramolecular 3′,5′-cyclicphosphates[39], than the nucleoside-5′ phosphates which can be used as monomers in the polymerisation of deoxyribonucleotides. Thus the addition of 2′,5′-*O*-diacetyluridine-3′ phosphate to 2′-*O*-acetyluridine-3′ phosphate should suppress side-reactions owing to the acceleration of the formation of oligonucleotide chains having 5′-*O*-acetyl terminal groups[13]. However, these measures have not yet led to a significant change in the results (Table 1).

For the cleavage of pyrophosphates, which are also formed during the polymerisation, the reaction mixture is first treated with aqueous pyridine[13] and then with an excess of acetic anhydride in dry pyridine[40].

In extending the number of monomers it was shown that the standard conditions under which polymerisation is carried out are not always acceptable: in some instances the starting materials are insoluble in pyridine, in others the pyridinium salts formed during dissolution are precipitated. To avoid these difficulties, the medium used was a mixture of pyridine and formamide with Dowex-50 in the pyridinium form[29], a mixture of dimethylformamide and pyridine with Dowex-50 in the pyridinium form[12], or the monomer was changed into another, soluble form (for example, TppT was used instead of Tp)[22].

TABLE 1.

Polynucleotides*	Yield of polynucleotides**, %						
	N = T			N=(d)-A	N=(d)-C	N = A	N = U
	pT	polymerisation of Tp: DCC	polymerisation of Tp: trichloroacetonitrile				
Linear:							
Np		0.1	6.3			26.0	8.3
NpNp	5.83	0.3	4.2	18.31	10.8	20.3	8 3
NpNpNp	9.24	3.5		14.20	15.0	12.0	5.5
NpNpNpNp	8.97	3.4	5.7	12.0	11.35	7.0	4.7
NpNpNpNpNp	7.8	4.0		9.0	8.20	4.2	2.3
$Np(Np)_4Np$	6.70			4.95	5.33		1.1
$Np(Np)_5Np$	5.20			3.09	3.14		1.0
$Np(Np)_6Np$	4.12			1.5	1.84		0.76
$Np(Np)_7Np$	3.19						0.08
$Np(Np)_8Np$	2.57						0.06
$Np(Np)_9Np$	2.07						
$Np(Np)_{10}Np$	1.52						
Higher***	4.71				3.65	3.8	
Cyclic:							
NpNp	10.0	16.5	9.5		32 ****	3.0	6.7
NpNpNp	3.45	4.0		8.29	2.44	7.0	5.0
NpNpNpNp	1.42	3.1			1.05		
NpNpNpNpNp and unidentified	1.58	3.3					

* N = nucleoside residue.

** The yields are given on the basis of the spectrophotometric results.

*** Unidentified.

**** Yield of cyclodinucleotide + (d)–pC.

§ See the first note on p. 531.

Linear polynucleotides with a small degree of polymerisation (for example, up to hexa-) are isolated, as already noted, by use of ion-exchange chromatography on DEAE-cellulose in the carbonate or hydrogen carbonate form at pH 8–9, by eluting the corresponding fractions with triethylammonium hydrogen carbonate. Polynucleotides of higher molecular wieghts are purified by repeatedly chromatographing appropriate fractions on DEAE-cellulose in the chloride form and using linear gradient elution with solutions of lithium chloride or lithium acetate. The homogeneity of the polynucleotides obtained is confirmed by enzymic treatment and also by chromatography and electrophoresis on paper.

III. HOMOPOLYNUCLEOTIDES

1. Deoxyribopolynucleotides.

Polymers have now been obtained of all four fundamental deoxyribonucleotides. The greatest number of work relates to the production of thymidine polynucleotides. (I), (II)(a) and also TppT have been used as monomers[22]. The reagents which activate the monomers are DCC, trichloroacetonitrile[24], and diethyl 1-ethoxy-2-ethoxy-carbonylvinyl phosphate[23]. The best results were given by polymerisation of (II)(a) mixed with pT-OAc (3 : 1) in anhydrous pyridine in the presence of DCC (in Table 1).

The polymerisation of (II)(b) mixed with (d)-$pA^{Bz}OBz$ (3 : 1) and (II)(c) mixed with (d)-$pC^{Ac}OAc$ (3 : 1) under standard conditions (anhydrous pyridine, DCC, room temperature), with subsequent removal of the protective groups led to a mixture of polymers, of which the constituent with the highest molecular weight had a degree of polymerisation of 8 (higher members were not identified) (see Table 1). The use of this method for obtaining deoxyguanosine polynucleotides met with several difficulties[29]. The monomer (II)(d) was almost insoluble in dry pyridine. Some other protected derivatives of guanosine-5′ phosphate containing more lipophilic groups were therefore obtained: *N*-benzoyl-, *N*-naphthyl, and *N*-di-*p*-methoxytrityl-deoxyguanosine-5′ phosphate. Of these, the last compound is sufficiently soluble in anhydrous pyridine. However, its polymerisation under standard conditions did not gave satisfactory results: 55% of the original monomer, 8% of a dinucleotide, and only 12% of higher oligonucleotides were obtained. Other studies also showed that the size of the protective groups affects the formation of the internucleotide bond[31,41], therefore the derivatives obtained could not be used as monomers. An attempt was made to use for polymerisation salts of *N*-acetylguanosine-5′ phosphate and stronger bases (tri-n-decylamine), which are more soluble in pyridine than the pyridinium derivatives. As the use of DCC as a polymerising agent does not permit such strong bases as trialkylamines to be used[11,42], the salt mentioned was polymerised in the presence of *p*-toluenesulphonyl chloride.

However, the polymerisation mixture was very complex and many of its components could not be identified. The most successful was an attempt to polymerise (II)(d) mixed with dimethylformamide and pyridine by adding the pyridinium form of Dowex-50 to create the proton concentration needed for carrying out the reaction in the presence of DCC[11]. Nevertheless, deoxyguanosine polynucleotides could not be obtained pure and identified by the usual means, since on treating the polymerisation products with concentrated ammonia to remove the *N*-acetyl groups, polymers were obtained which form, according to the results of enzymic studies, high-molecular-weight aggregates with an ordered secondary structure[29,43]. Therefore subsequently the polymerisation mixture (without removal of the protective groups) was separated on a column of DEAE-cellulose in the hydrogen carbonate form under conditions which excluded the detachment of *N*-acetyl groups. Table 2 gives the results.

2. Ribopolynucleotides

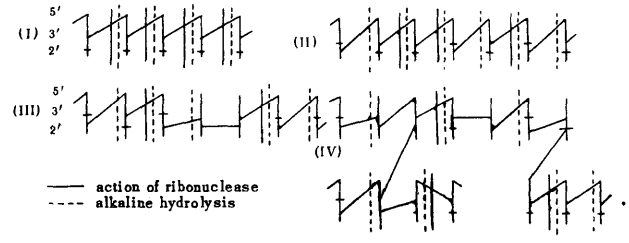
The preparation of ribopolynucleotides is a more complex task because the corresponding monomers are less accessible and the reaction mixture contains more components.

Michelson[44] proposed to obtain ribonucleotides by using ribonucleoside-2′,3′ cyclic phosphates as the monomers. Preliminary work with glycerol-2(or 1) phosphate showed that on treatment of the monomer with diphenyl phosphorochloridate or tetraphenyl pyrophosphate[45], a polyglycerophosphoric acid is readily obtained through the intermediate stage of P^1-glycerol-2(1)-P^2-diphenyl pyrophosphate, which is rapidly converted into glycerol-1,2 cyclic phosphate. Similar treatment of adenosine-2′(3′) phosphate with diphenyl phosphorochloridate gives initially adenosine-2′,3′ cyclic phosphate[46], subsequent polymerisation of which leads to a polyadenylic acid with a chain length varying from that of a dinucleotide to polynucleotides containing up to 15 monomeric units.

The method is easily extended to the synthesis of polymers of guanosine-2′(3′) phosphate, uridine-2′(3′) phosphate[47], pseudouridine-2′(3′) phosphate[36]. A similar polymerisation of cytidine-2′(3′) phosphate led to a polymer stable against treatment with alkalis and diesterases, and probably containing phosphoramide bonds between the 2′(3′)-phosphate groups and the 6-amino-group of the cytosine. Selective acetylation of the amino-group in cytidine-2′,3′ cyclic phosphate and treatment of the product with diphenyl phosphorochloridate followed by removal of the acetyl groups allowed polycytidylic acids with chains of various lengths to be obtained[36].

In addition polymers have been obtained from analogues of nucleotides, including *N*-methyluridylic, 5-bromouridylic, and 5-iodouridylic acids[48]. The mean chain lengths of the polymers thus obtained amounted to 10–12 nucleotide residues. Study of the properties of these polymers showed that they contain ~50% $C_{(2')}$–$C_{(5')}$ internucleotide bonds.

Schramm and coworkers[38] reported the preparation of polyadenylic, polycytidylic, polyguanylic, polyuridylic, and polythymidylic acids of high molecular weight (15 000–50 000). To avoid intramolecular cyclisation these authors recommend the use of reaction mixtures as concentrated as possible, by mixing the nucleotides to be polymerised directly with a viscous ester of a polyphosphoric acid. However, the work does not contain a direct proof that the polynucleotides formed contain only $C_{(3')}$–$C_{(5')}$ bonds. At the same time, Kochetkov and coworkers[49] showed that the condensation of a nucleoside-2′(3′) phosphate under the conditions specified can lead theoretically to the formation of compounds of the following types:



Polymers of type (I) should give only mononucleotides on treatment with ribonuclease, polymers of type (II), di- and oligo-nucleotides. Alkaline hydrolysis leads predominantly to mononucleotides with (II) and predominantly to mononucleotides with (III), only with (IV) do both enzymic and alkaline hydrolyses lead to a complex mixture consisting chiefly of oligonucleotides. The results of treating polynucleotides obtained by Schramm's method with ribonuclease and 2*N* KOH suggest that bonds of the non-native type predominate in these polynucleotides.

Khorana and coworkers obtained adenine[32] and uridine[13,33] polynucleotides by taking (V)(a) and (V)(b) as monomers. They treated a mixture of (V)(a) and *N*, 2′, 5′-triacetyladenosine-3′ phosphate (3 : 1) with DCC in dry pyridine by the standard method for 6 days and after the usual treatment (cleavage of the pyrophosphate bonds and removal of the protective groups), the product was separated on DEAE-cellulose (see Table 1). A mixture of (V)(b) and 2′,5′, *O*-diacetyluridine-3′ phosphate was polymerised in a similar way, and after repeated chromatography on DEAE-cellulose polynucleotides up to decauridylic acid inclusive were obtained in an individual state (see Table 1). However, many side-products remained to be identified. They probably contain chiefly two series of compounds: homologous oligonucleotides with 2′3′-cyclic phosphate terminal groups and with 3′-phosphate or 2′,3′-cyclic phosphate groups on one end of the chain and *C*-5′-pyridine group on the other. The presence of a large number of side-products in the polymeric mixture emphasises the additional difficulty of work with ribonucleotides.

TABLE 2.

Polynecleotide	Yield, %
N-Acetylguanosine-dinucleotide	15.4
N-Acetylguanosine-trinucleotide	9.4
N-Acetylguanosine-tetranucleotide	5.2
N-Acetylguanosine-pentanucleotide	4.9
Higher	9.78
Cyclotrinucleotide	26.8

IV. HOMOPOLYNUCLEOTIDES WITH DIFFERENT TERMINAL NUCLEOTIDES

Khorana and coworkers[26] have proposed a general method for obtaining homopolynucleotides having another nucleotide

TABLE 3.

Polynucleotides	Yield, %		
	chromatography on DEAE-cellulose	repeated chromatography	
		homo-	mixed
pTpT + (d)-pTpC	13	35	65
pTpTpT + (d)-pTpTpC	15.3	46	54
pTpTpTpT + (d)-pTpTpTpC	9.8	42	58
$pT(pT)_3pT$ + (d)-$pT(pT)_3pC$	8.25	41	59
$pT(pT)_4pT$ + (d)-$pT(pT)_4pC$	5.05	41	59
$pT(pT)_5pT$ + (d)-$pT(pT)_5pC$	3.52	41	59
$pT(pT)_6pT$ + (d)-$pT(pT)_6pC$	2.14	41	59
$pT(pT)_7pT$ + (d)-$pT(pT)_7pC$	1.38	37	63

TABLE 4.

Polynucleotides	Yield, %	Polynucleotides	Yield, %
(d)-TpC	28.0	(d)-pCpCpCpC	4.6
(d)-pTpCpC + cyclo-(d)-pCpC	14.0	(d)-TpCpCpCpCpC	3.3
(d)-pCpC	6.1	(d)-pCpCpCpCpC	4.0
(d)-TpCpCpC	8.7	(d)-$Tp(Cp)_3C$	2.3
(d)-pCpCpC + cyclo-(d)-pCpCpC + unidentified	9.7	(d)-$pC(pC)_4pC$	2.2
(d)-TpCpCpCpC	7.5	Higher	0.9

at the 3′ end of the chain. The method consists of the addition to a monomer (nucleoside-5′ phosphate) of a completely substituted derivative of another nucleotide. In particular, a mixture of thymidine-5′ phosphate and *NO*-3′-diacetyldeoxycytidine-5′ phosphate was subjected to polymerisation under standard conditions. Table 3 gives the results.

In the same laboratory[12] it was proposed to obtain homopolynucleotides with a distinctive terminal nucleotide by treating the mixture after polymerisation of the nucleoside-5′ phosphate with the appropriate protected nucleoside having a free 3′-OH group, in the presence of a condensing agent. Thus a mixture of *N*-anisyldeoxycytidine polynucleotides immediately after polymerisation was treated with an excess of 5′-*O*-dimethoxytritylthymidine and DCC in anhydrous dimethylformamide. After removing the protective groups and chromatographing the mixture on DEAE-cellulose the results in Table 4 were obtained. Attempts to treat in a similar manner not a mixture of polymers but a purified oligonucleotide showed that best results are obtained in working with a mixture.

V. POLYNUCLEOTIDES WITH A RANDOM DISTRIBUTION OF PURINE AND PYRIMIDINE BASES ALONG THE CHAIN

Polynucleotides consisting of different nucleotide units with a random distribution of purine and pyrimidine bases were obtained by Michelson[47] from various mixtures of nucleoside-2′,3′ cyclic phosphates. However, the copolymers obtained are not of great interest, as they contain, in addition to the $C_{(3')}-C_{(5')}$ internucleotide bonds, ~50% $C_{(2')}-C_{(5')}$ bonds, and therefore we will not consider them in detail. The longest polynucleotide chain obtained in this way did not exceed 10–12 nucleotide units.

Mixed polynucleotides were also obtained by condensing a mixture of nucleoside-2′(3′) phosphates in the presence of a polyphosphate ester[49]. Table 5 gives information on the reagent proportions, reaction conditions, yields, and compositions of the polymers. As noted above, non-native bonds predominate in these polynucleotides.

VI. POLYNUCLEOTIDES WITH REGULARLY ALTERNATING PURINE AND PYRIMIDINE BASES

At present we know of only three works on the preparation of polynucleotides with regularly alternating bases. In the first, on the polymerisation of dinucleotides[30] the monomer was (III)(b). Interest in studying polynucleotides with such a sequence was stimulated by the knowledge that in some types of crabs there is found a DNA containing predominantly two of these nucleotides in alternating sequence[50-52]. The polymerisation of (III)(b) was carried out in the presence of DCC under standard conditions and after the usual treatment the results shown in Table 6 were obtained.

The study of the ultraviolet absorption (260 nm) as a function of temperature was made in the presence of 1 M NaCl (pH 7) and showed that the polynucleotides obtained have a secondary structure. It was preliminarily found that the dodecanucleotides and the fraction containing higher nucleotides act as seeds for the catalysable DNA-polymerase [DNA nucleotidyltransferase (Ed. of Translation)] synthesis of large T-(d)-A copolymers.

The polymerisation of (III)(a) was effected in a mixture of dimethylformamide, pyridine, and the pyridinium form of Dowex-50 in the presence of DCC. The resulting mixture, after the standard treatment, was separated on DEAE-cellulose in the presence of 7 M urea[53]. It should be noted that in both instances a large amount of a cyclodinucleotide is formed and the degree of polymerisation is not high (Table 7).

Finally, Schramm and coworkers recently published a brief communication[34] on the polymerisation of ApGpCp and AdGpUp in ethyl tetrametaphosphate and trisdimethylaminophosphate. The polymers were separated on Sephadex G-75. Information was not given about the composition of the mixture, degree of polymerisation, etc.

In conclusion, it must once again be emphasised that the methods of chemical synthesis of polynucleotides are now at present quite imperfect and require improvement and further development. In particular, one of the principal

TABLE 5.

Reagents				Reaction conditions		Yield of polymer, %	Composition of bases (A/C or A/U)
AMF, mM	UMF, mM	CMF, mM	PPE*, g	°C	h		
—	2.5	—	24	70—80	40	20—25	—
0.15	—	0.31	3.5	70—75	60	12.2	1:4.5
0.23	—	0.23	3.5	70—75	60	12.5	1:3.3
0.31	—	0.15	3.5	70—75	60	7.6	1:1.4
0.15	0.31	—	4.0	60	30	8.9	1:1.91
0.23	0.23	—	4.0	60	30	5.8	1:0.95
0.31	0.15	—	4.0	60	30	3.2	1:0.58

* PPE = polyphosphate ester.

TABLE 6.

Polynucleotides	Yield, %
Cyclo-(d)-pTpA	36.3
(d)-pTpA	22.6
(d)-pTpApTpA	10.7
(d)-pTpApTpApTpA	5.0
(d)-pTpApTpApTpApTpA	2.0
Higher (~50% decanucleotide)	1.1

TABLE 7.

Polynucleotides	Yield, %
(d)-pCpG	23.2
Cyclo-(d)-pCpG	16.8
(d)-(pCpG, O, pCpG)	18.0
(d)-pCpGpCpG	8.75
(d)-(pCpG, O, pCpGpCpG)	3.43
(d)-pCpGpCpGpCpG + higher + + pyrophosphates	8.75

deficiences in the method is that despite many attempts, it has so far proved impossible to obtain and isolate polynucleotides containing more than 12 nucleotide residues and the yield of the longest of the identified polynucleotides does not exceed 1–2%. In this connection the main tasks of the chemical synthesis of polynucleotides consists in increasing the degree of polymerisation and yield of polymers and also in the development of methods of separating the high-molecular-weight oligonucleotides. It is quite probable that a more careful study of the kinetics and thermodynamics of the polycondensation reactions, a choice of new condensing agents, and the introduction into the "monomer" of more active groups which participate in the reaction are needed. A new approach to the chemical synthesis of polynucleotides should make available polynucleotides with a known succession of purine and pyrimidine bases in the polynucleotide chain, which is known to be impossible in the enzymic synthesis of polynucleotides. This allows the solution on the molecular level of the problems connected with the structure and functions of the nucleic acids, the mechanism of protein synthesis, etc.

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EXPERIMENT AND THE ELECTRONIC THEORY OF CATALYSIS†

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1. INTRODUCTION

In the present lecture I shall endeavour to discuss the electronic theory of catalysis from the point of view of the experimentalist who wishes to apply its results rather than from the point of view of the theoretician who is contributing to its development. I shall be talking of the experimental material on which the theory is based: that which has given rise to the theory and that which, in turn, stems from the theory.

Some simple relationships exist in general (and in the field of catalysis, in particular) between physical theory and experiment which can be expressed by the trite statement that theory divorced from experiment is effectively meaningless whilst experiments lacking a theory are blind. Experiment is the fertile soil in which theory can grow; theory, like a theatrical spotlight, throws light on experiment.

The electronic theory of catalysis has a twofold aim: on the one hand, to reveal the elementary mechanism of the catalytic act and at the same time to discover the relationship between the chemisorption and the catalytic properties of a surface, and on the other hand to relate those characteristics to the electronic properties of the crystal as a whole. The electronic theory of catalysis does not, in general, conflict with other contemporary theories of catalysis: it differs from them only in its approach to the problem. It is concerned with the elementary (electronic) mechanism of the phenomenon, and it tackles problems in catalysis from just this point of view.

The electronic theory of catalysis proved to be the gate through which contemporary solid state theory and the theory of the chemical bond, together with their associated quantum mechanics and quantum statistics, were able to enter the field of catalysis. The history of science has no comparable examples to offer of an invasion by physics of the territory of chemistry. Consider, for instance, the theory of the chemical bond, which even 50 years ago consisted of nothing more than dashes denoting valencies. Quantum mechanics has given those dashes a physical content, thus uncovering the physical nature of chemical forces.

We can distinguish two aspects of the interplay between the electronic theory of catalysis and experiments, which the present article aims to discuss.

Firstly, the theory provides an explanation of known experimental facts. These can be *old* facts, fitting more or less well into old theories, on which the electronic theory throws new light. Or they can be *new* facts, transcending the scope of the older theories, and deriving some theoretical interpretation, for the first time, from the electronic theory.

Secondly, the electronic theory leads to a number of experimental predictions. Like any other theory, it contains explicit or implicit rules and relationships which can be (and should be) identified and tested experimentally.

It is useful and interesting to gather together and analyse the basic experimental material which is satisfactorily interpreted by means of the electronic theory and which can therefore be entered on the credit side.

At the same time, it is particularly interesting to collect and analyse all the corollaries of the electronic theory still awaiting experimental confirmation. Such is the aim of the present paper.