Studies on Polynucleotides. LXIII.[1] Deoxyribopolynucleotides Containing Repeating Trinucleotide Sequences (5). The Polymerization of Protected Deoxyribotrinucleotides[2]

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Abstract: The synthesis, isolation, and characterization of the 13 series of deoxyribopolynucleotides containing repeating trinucleotide sequences listed in Table I are reported. The general method used involved the polymerization of suitably protected deoxyribotrinucleotides with aromatic sulfonyl chlorides as the condensing agents. After removal of the protecting groups, the oligomers of the trinucleotides were purified by a combination of the techniques of gel filtration on Sephadex G-50 columns, anion-exchange chromatography, and paper chromatography.

Considerations for the synthesis of deoxyribopolynucleotides containing repeating trinucleotide sequences have been outlined separately.[3] The present paper records the synthesis, isolation, and characterization of 13 (Table I) out of a maximum of 20 possible

Table I. Synthetic Deoxyribopolynucleotides with Repeating Trinucleotide Sequences[a]

d-pA(pApCpA)$_n$pApC, n = 1–5	d-pC(pCpTpC)$_n$pCpT, n = 1–4
d-pT(pTpGpT)$_n$pTpT, n = 1–4	d-pG(pGpApG)$_n$pGpA, n = 1–4
d-pT(pApCpT)$_n$pApC, n = 1–5	d-pG(pGpTpG)$_n$pGpT, n = 1–4
d-pT(pApGpT)$_n$pApG, n = 1–5	d-pC(pCpApC)$_n$pCpA, n = 1–4
d-pA(pTpCpA)$_n$pTpC, n = 1–4	d-pA(pApGpA)$_n$pApG, n = 1–4
d-pA(pTpGpA)$_n$pTpG, n = 1–5	d-T(pTpCpT)$_n$pTpC[b], n = 1–3
d-pC(pGpApC)$_n$pGpA, n = 1–4	
d-pC(pGpTpC)$_n$pGpT, n = 1–4	

[a] The synthetic polynucleotides are grouped in sets of two to show their complementary base-pairing relationship. [b] The synthesis of this complementary series containing repeating thymidylylthymidylyldeoxycytidylate sequences has been described previously.[4]

series of homologous short-chain deoxyribopolynucleotides containing repeating trinucleotide sequences, excepting the four homopolymers. Together with the previously described synthesis of deoxyribopolynucleotides containing the repeating sequence thymidylylthymidylyldeoxycytidine,[4] the polynucleotides listed in Table I provide the required templates for seven double-stranded DNA-like polymers containing repeating trinucleotide sequences.

Methods for the polymerization of suitably protected deoxyribomononucleotides were developed several years ago.[5] A comparative study of reagents for polymerization showed dicyclohexylcarbodiimide to be the best reagent[5f] and, therefore, the same reagent was used for the polymerization of suitably protected dinucleotides so as to form deoxyribopolynucleotides with repeating dinucleotide sequences.[6] The polymerization mixtures in these experiments were heterogeneous and the extent of polymerization was rather limited. A very large number of side products were formed, in addition to substantial amounts of oligo- and polynucleotides containing pyrophosphate linkages. As a result, extensive purification was required in the isolation of pure desired polynucleotides. Therefore, in further work encompassing the synthesis of polynucleotides with repeating trinucleotide sequences, the approach involving stepwise addition of mononucleotides to a growing polynucleotide chain[4,7,8] was used.

More encouraging results recently have been obtained in condensation of preformed oligonucleotide blocks using aromatic sulfonyl chlorides as the condensing agents.[9–11] These reagents therefore appeared to be superior to dicyclohexylcarbodiimide in providing adequate activation of the phosphomonoester groups in protected di- and trinucleotides. Because of these results and the magnitude of the total synthetic task, where stepwise procedures have to be used, the application of the polymerization approach to the synthesis of deoxyribopolynucleotides containing repeating trinucleotide sequences was further investigated. This method has in fact been used in the synthesis of all of the polynucleotides listed in Table I.[12] The present

(1) Paper LXII: S. A. Narang, T. M. Jacob, and H. G. Khorana, *J. Am. Chem. Soc.*, **89**, 2158 (1967).

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(7) S. A. Narang and H. G. Khorana, *ibid.*, **87**, 2981 (1965).

(8) S. A. Narang, T. M. Jacob, and H. G. Khorana, *ibid.*, **87**, 2988 (1965).

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(10) H. Kössel, H. Büchi, and H. G. Khorana, *ibid.*, **89**, 2185 1967.

(11) E. Ohtsuka and H. G. Khorana, *ibid.*, **89**, 2195 (1967).

(12) The system of abbreviations for specification of protected and unprotected polynucleotides is as has been defined in an accompanying paper.[1] In the present work and in the following paper dealing with rather large polymers containing repeating sequences, further abbrevia-

paper documents the total experimental work on polymerization, isolation, and characterization of these deoxyribopolynucleotides. The preparation of suitably protected deoxyribotrinucleotides, which served as the starting materials in this work, has been described in the preceding paper.[1]

The general method for polymerization used a mixture of the protected trinucleotide (*e.g.*, d-pTpTpG^{Ac})[12] and the corresponding 3′-O-acetyl derivative (*e.g.*, d-pTpTpG^{Ac}-OAc) in a molar ratio of approximately 4:1. This technique, as found previously,[5c] reduces the extent of intramolecular reaction to form the cyclotrinucleotides. The medium of reaction used for polymerizations was dry pyridine; homogeneous solutions were obtained invariably at all concentrations when the starting materials were used as their tri-*n*-hexylammonium salts. Mesitylenesulfonyl chloride (MS) or triisopropylbenzenesulfonyl chloride (TPS) were used as the reagents. An excess (3–4 molar proportions for every phosphoryl dissociation) of the reagents was used and the time of reaction was kept to a minimum (1.5–2 hr at room temperature) to reduce the extent of side reactions. In order to promote *linear* polymerization and to increase its rate, the reaction mixtures were concentrated further soon after the addition of the condensing agent to the anhydrous pyridine solutions.

The standard work-up in polynucleotide condensations usually requires prolonged aqueous pyridine treatment as the first step. In the early phase of the present work, especially in polymerization of the trinucleotides, d-pA^{Bz}pA^{Bz}pG^{Ac} and d-pA^{Bz}pA^{Bz}pC^{An}, evidence for some loss of the N-benzoyladenine group was obtained. This depurination evidently increased greatly the number of side products in the polymeric mixtures. It is our impression that this depurination occurred at least partly during the aqueous pyridine treatment and was caused by the presence of large amounts of pyridinium hydrochloride and pyridinium salts of the sulfonic acids generated from the condensing agents. As a precaution an excess of trialkylamine was routinely added at the start of the aqueous pyridine treatment of the polymerization mixture. Another precaution that was taken during the preparation of the polymerization reaction mixtures was to ensure that sufficient amounts of pyridine remained after concentration of the initial anhydrous pyridine solution of the reactants. With these precautions, the extent of depurination was reduced to undetectable levels.

The methods used in some of the early work for separation and purification of the polymers (oligomers of the trinucleotides) included chromatography on DEAE-cellulose anion-exchange columns followed by purification by peparative paper chromatography. Thus the polymers containing repeating d-pTpApC sequences were separated on a DEAE-cellulose column in the carbonate form (Figure 1) and those containing the repeating d-pTpApG sequences were separated on a DEAE-cellulose (chloride) column in the presence of 7 *M* urea. In the greater part of the work, the methods used included initial separation of the polymeric mixture, roughly as a function of size, on Sephadex G-50 columns, and this was followed by extensive paper chromatography. Direct paper chromatography alone of the polymeric mixtures gave invariably a series of well-resolved ultraviolet-absorbing bands. These contained compounds up to the nonanucleotides. Compounds higher than this size were close to the origin and were not resolved by a single-step chromatography of all of the mixture. A generally satisfactory sequence of steps for separation of the products, therefore, involved separation on a Sephadex G-50 column followed by extended paper chromatography of the completely or partially resolved peaks corresponding to materials of different chain length.

While side products were present in each series (see below), the major products always corresponded to the trinucleotide, the hexa- and nonanucleotides, and the successively higher oligomers of the trinucleotide. Correlation of the different peaks of ultraviolet-absorbing products from Sephadex G-50 columns and their chain length was often evident from the pattern of elution. The behavior on paper chromatograms (in *n*-propyl alcohol–ammonia–water solvent) of the different oligomers also readily permitted allocation of chain length to the different members. Thus, in each series, the R_f values of the tri-, hexa-, nona-, dodeca-, and higher polynucleotides showed an orderly decrease by a factor of approximately three. The required products thus recognized were eluted, and for further purification they were incubated with bacterial alkaline phosphatase to remove the 5′-phosphomonoester groups and the resulting products were again subjected to chromatography in the *n*-propyl alcohol–ammonia–water system. Removal of the phosphomonoester group rather consistently doubled the R_f in this solvent, and this type of behavior further supported the identification. Again this series of oligomers lacking the phosphomonoester groups showed a regular decrease in R_f's on chromatography. Any side products still present at this stage were removed by final chromatography in the buffered acidic solvent, isobutyric acid–ammonia–water.

Full documentation of the methods of isolation of the homologous polynucleotides in each series is described in the Experimental Section. Selected members (up to the nonanucleotide) in each series were characterized by enzymatic degradation with spleen phosphodiesterase to the constituent mononucleotides and terminal nucleosides (results in Table II). Characterization of members selected from each series in this way further supported the assignment of chain length to the higher oligomers made on the basis of chromatographic mobilities.

From previous experience with polymerization reactions, the side products to be expected would be of the following types: (1) cyclotrinucleotide and perhaps cyclohexanucleotides; (2) pyrophosphates formed by linkage of the trinucleotide and higher polynucleotides to each other through their phosphomonoester groups; (3) oligo- and polynucleotides containing phospho-

tions as introduced in an earlier paper [H. G. Khorana and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **83**, 675 (1961)] are also used. Accordingly, the polynucleotides listed in Table I are abbreviated so as to show the repeating trinucleotide unit but at the same time to show the nucleoside units of the polynucleotide chain which bears the 5′-phosphate and the 3′-hydroxyl end groups. Thus the nonanucleotide, pApApCpApApCpApApC, cannot *strictly* be abbreviated to (pApApC)$_3$ but is appropriately abbreviated to pA(pApCpA)$_2$pApC. The same system was used previously in designation of deoxypolynucleotides containing repeating dinucleotide sequences.[6c] It is also extended to the polymers described in the succeeding papers, which contain repeating tetranucleotide sequences.

Table II. Spleen Phosphodiesterase Degradation of Deoxyribopolynucleotides Containing Repeating Trinucleotide Sequences

Compd	OD_{260} units degraded	Products nucleotides: nucleoside	Molar ratio of products—Found	Molar ratio of products—Theor
d-TpApCpTpApC	4.5	Tp:d-Ap:d-Cp:d-C	2:2:1:1	2:2:1:1
d-TpApCpTpApCpTpApC	6.0	Tp:d-Ap:d-Cp:d-C	3.1:3:2:1	3:3:2:1
d-TpApGpTpApGpTpApG	5.5	Tp:d-Ap:d-Gp:d-G	3.05:3:2:1	3:3:2:1
d-ApTpCpApTpCpApTpC	6.2	d-Ap:d-Tp:d-Cp:d-C	3.2:3:2.1:1	3:3:2:1
d-ApTpGpApTpGpApTpG	5.0	d-Ap:d-Tp:d-Gp:d-G	2.9:3:2:1	3:3:2:1
d-TpTpGpTpTpGpTpTpG	6.0	Tp:d-Gp:d-G	5.9:2.1:1	6:2:1
d-ApApCpApApCpApApC	5.0	d-Ap:d-pC:d-C	6.2:2.0:1	6:2:1
d-CpGpApCpGpApCpGpA	5.2	d-Cp:d-Gp:d-Ap:d-A	3.1:3.0:2:1	3:3:2:1
d-CpGpTpCpGpTpCpGpT	6.1	d-Cp:d-Gp:Tp:T	3.05:3.1:2:1	3:3:2:1

monoester groups at both terminals; and (4) polynucleotides containing a quaternary pyridinium group at 5′ carbon of terminal nucleosides. Cyclic trinucleotides were invariably encountered, and these were characterized by their high paper chromatographic mobility and by their resistance to the phosphomonoesterase. In the present polymerization experiments, pyrophosphates were present in very minor amounts. This general conclusion was drawn from the fact that the symmetrical pyrophosphate formed from two molecules of the trinucleotide, recognized by its presence on chromatograms (solvent D) ahead of the hexanucleotide, accounted for a very small portion of the total unreacted trinucleotide. It is reasonable to conclude that this result was due to the use of an excess of the sulfonyl chloride reagent. The phosphomonoester groups in oligo- and polynucleotides were probably present as mixed anhydrides with the sulfonic acids rather than as pyrophosphates.[13] While side products of type 3 and 4 were probably present, they were not investigated. However, it is certain that there was no difficulty in ensuring that the desired homologous polynucleotides were free from them. The sequence of steps used routinely in purification would have removed both classes of side products. Thus, for example, polynucleotides having phosphomonoester groups at both terminii would be removed upon paper chromatography after phosphomonoesterase treatment, the increase in their mobility after the enzymatic step being much greater than that obtained for the polynucleotides bearing only one phosphomonoester group.

Most of the sets of complementary deoxyribopolynucleotides herein described have been used successfully as templates for DNA polymerase, and the products formed in the presence of the deoxyribonucleoside 5′-triphosphates of the four bases have been characterized as DNA-like polymers containing strictly repeating trinucleotide sequences present in the chemically synthesized short-chain templates.[14–16] In turn, the DNA-like polymers have been used as templates for DNA-dependent RNA polymerase, and the resulting single-stranded ribopolynucleotides have again been shown to contain the repeating trinucleotide sequences. Finally, use of the single-stranded ribopolynucleotides as messengers in the cell-free protein-synthesizing system has already provided new as well as confirmatory information on the structure of the genetic code such that most of the current codon assignments can be regarded as proven.[15–17]

(13) The degradation of dithymidine pyrophosphate by treatment with excess mesitylenesulfonyl chloride in dry pyridine has been demonstrated previously [T. M. Jacob and H. G. Khorana, *J. Am. Chem. Soc.*, **86**, 1630 (1964)].

(14) R. D. Wells, T. M. Jacob, H. Kössell, A. R. Morgan, S. A. Narang, E. Ohtsuka, and H. G. Khorana, *Federation Proc.*, **25**, 404 (1966).

(15) H. G. Khorana in "Proceedings of the 3rd Meeting of European Biochemists, Warsaw, 1966," Academic Press Inc., New York, N. Y., in press.

(16) H. G. Khorana, H. Büchi, H. Ghosh, N. Gupta, T. M. Jacob, H. Kössel, R. Morgan, S. A. Narang, E. Ohtsuka, and R. D. Wells, *Cold Spring Harbor Symp. Quant. Biol. (June 1966)*, in press.

Experimental Section

General Methods and Materials. Paper chromatography and electrophoresis were performed as described earlier.[1]

Reagent grade pyridine was distilled and dried over calcium hydride or Molecular Sieve or beads (4A) from Linde Co. for several weeks. Sephadex G-50 and G-75 were purchased from Pharmacia, Uppsala, Sweden. All evaporations were carried out using a rotary apparatus under reduced pressure.

Enzymic degradation of the synthetic products was carried out by using bacterial alkaline phosphomonoesterase, spleen phosphodiesterase, and venom phosphodiesterase as described previously.[5d]

General Method for the Polymerization of Trinucleotides Using Mesitylenesulfonyl Chloride. The protected trinucleotide was converted to tri-*n*-hexylammonium salt by treating its aqueous pyridine solution with a slight excess of tri-*n*-hexylamine. The mixture was reduced to a gum by repeated evaporation of added dry pyridine. The excess of tri-*n*-hexylamine was removed at this stage by washing with ether and removing the ether by decanting.

To an anhydrous mixture of tri-*n*-hexylammonium salts of 3′-O-acetyl N-protected trinucleotide (20%) and N-protected trinucleotide (80%) in dry pyridine (1–2 ml) was added mesitylenesulfonyl chloride (10–15 equiv as based on the total trinucleotide) inside a drybox. The reaction mixture was further concentrated *in vacuo* to a viscous solution (0.5–1.0 ml) with gentle shaking and the sealed reaction mixture was kept at room temperature in the dark for 1.5–2 hr. Aqueous pyridine (2 ml) was then added under cooling, followed by an excess (0.5–1 ml) of triethylamine, and the resulting solution was allowed to stand at room temperature overnight. For removal of anisoyl, benzoyl, and acetyl groups from the amino groups of the oligonucleotides, the aqueous pyridine solution was treated with an excess (two to three times the volume of pyridine) of concentrated ammonium hydroxide for 2 days at room temperature.

Isolation of Oligonucleotides. (A) Separation by Preparative Paper Chromatography. After removal of the protecting groups, a portion (~150 OD_{260} units) of the reaction mixture was applied to a 9-in.-wide strip of either Whatman No. 40 or 1 paper and was chromatographed in solvent D for 3 days. Many ultraviolet-absorbing bands with decreasing R_f's up to the origin were observed. The bands corresponding to members as high as the nonanucleotides were clearly separated; those corresponding to higher polynucleotides, which were close to or at the origin, were not resolved. The main bands were eluted with dilute ammonium hydroxide (0.1%) or with aqueous pyridine.

(B) Separation by Using Sephadex G-50 Column Chromatography. After the removal of protecting groups, a major portion of the concentrated polymerized mixture was applied to a Sephadex G-50 column preequilibrated with 0.1 *M* triethylammonium bi-

(17) A. R. Morgan, R. D. Wells, and H. G. Khorana, *Proc. Natl. Acad. Sci. U. S.*, **56**, 1899 (1966).

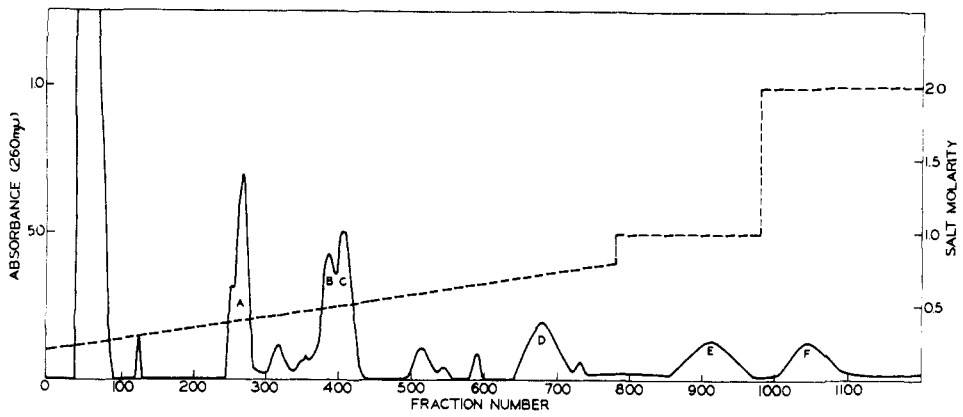

Figure 1. Chromatography of deoxyribopolynucleotides (hexanucleotide and higher) containing the repeating trinucleotide sequence thymidylyldeoxyadenylyldeoxycytidylate on a DEAE-cellulose (carbonate) column (88 × 2.5 cm). Elution was carried out with a linear gradient of triethylammonium bicarbonate, 0.2–0.6 *M*, 1.5 l. each, and 0.6–0.8 *M*, 1 l. each, and, finally, the column was washed with 1 *M* buffer. Fractions containing 8 ml were collected every 15 min.

carbonate buffer and eluted with the same buffer. Pooled fractions were concentrated, applied on Whatman No. 40 or 1 paper, and chromatographed in solvent D or C. Prolonged periods (5–10 days) were used for chromatography in the case of components higher than nonanucleotides. Major bands were eluted as described above.

General Methods for Purification and Characterization of Oligonucleotides. Each band for further purification was treated with bacterial alkaline phosphomonoesterase and the resulting product was rechromatographed successively in solvents D and C.

Polymerization of a Mixture of d-pA^{Bz}pA^{Bz}pC^{An} and d-pA^{Bz}pA^{Bz}pC^{An}-OAc. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pA^{Bz}pA^{Bz}pC^{An} (2000 OD_{260} units, 0.036 mmole) and d-pA^{Bz}pA^{Bz}pC^{An}-OAc (500 OD_{260} units, 0.0097 mmole) in dry pyridine (1 ml) was treated with mesitylenesulfonyl chloride (100 mg) under standard conditions. After usual treatment with aqueous pyridine (1 ml) and concentrated ammonium hydroxide, the oligonucleotides were isolated by paper chromatography. The R_f's and the yields of the homologous products are given in Table III.

Table III. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Sequence Deoxyadenylyldeoxyadenylyldeoxycytidylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group
Cyclo-d-pApApC	10.0	3.0	3.0
d-pApApC	30.0	2.5	4.0
d-pApApCpApApC	7.0	1.0	2.0
d-pA(pApCpA)$_2$pApC	2.0	0.42	0.95
d-pA(pApCpA)$_3$pApC	0.1	0.15	0.35
d-pA(pApCpA)$_4$pApC	3.0	...	0.17
d-pA(pApCpA)$_5$pApC		...	0.08
Higher homologs			

[a] R_f relative to the hexanucleotide, d-pApApCpApApC.

Polymerization of a Mixture of d-pTpA^{Bz}pC^{An} and d-pTpA^{Bz}pC^{An}-OAc and Isolation of the Resulting Polynucleotides. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pTpA^{Bz}pC^{An} (4500 OD_{280}, 0.13 mmole) and d-pTpA^{Bz}pC^{An}-OAc (1000 OD_{280}, 0.029 mmole) in dry pyridine (1 ml) was treated with mesitylenesulfonyl chloride (250 mg) under standard conditions for 1.5 hr. Aqueous pyridine (1.5 ml) was then added under cooling and the resulting solution was allowed to stand overnight at room temperature. The protected compounds up to hexanucleotide were separated by DEAE-cellulose (carbonate) column chromatography into well-defined peaks, and those corresponding to higher polynucleotides (1600 OD_{280}) were brought off the column with 1 *M* triethylammonium bicarbonate containing 10% alcohol and further (110 OD_{280}) with 1.8 *M* triethylammonium bicarbonate containing 20% alcohol. The mixture of the higher protected polynucleotides (800 OD_{280}) was treated with concentrated ammonia for 2 days, and the reaction mixture was concentrated in *vacuo* and applied on a DEAE-cellulose (carbonate) column. The elution pattern is shown in Figure 1 and the distribution of nucleotidic material is given in Table IV. The R_f's and the yields of the homologous products are given in Table V.

Table IV. Separation on a DEAE-Cellulose (Carbonate) Column of Higher Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Thymidylyldeoxyadenylyldeoxycytidylate (Elution Pattern in Figure 1)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	248–280	99	17.7	Hexanucleotide (95)
B	361–384	70	14 3	Nonanucleotide (50)
C	385–430	110	19.0	Nonanucleotide (75)
D	644–710	40	7.1	Dodecanucleotide (80)
E	870–960	30	5.4	Pentadecanucleotide (50)
F	1010–1100	15	2.7	Higher homologs

[a] Calculation of the yield: a known amount of the product from each peak was chromatographed in solvent D and the major ultraviolet-absorbing bands were eluted, treated with bacterial alkaline phosphatase, and rechromatographed in solvent D. The major bands of the desired products were eluted and estimated. The percentage given is the percentage of the final product relative to the original mixture from each peak applied on paper.

Table V. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Thymidylyldeoxyadenylyldeoxycytidylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group	R_f in solvent C[b] Without 5′-phosphate end group
Cyclo-d-pTpApC	7.1	3.4	3.4	
d-pTpApC	21.1	2.5	4.5	1.0
d-pTpApCpTpApC	18.8	1.0	2.0	0.66
d-pT(pApCpT)$_2$pApC	7.5	0.45	1.0	0.36
d-pT(pApCpT)$_3$pApC	1.8	0.18	0.35	0.23
d-pT(pApCpT)$_4$pApC	0.9	...	0.20	0.15
d-pT(pApCpT)$_5$pApC	3.0	...	0.08	0.09
Higher homologs				

[a] R_f with respect to the hexanucleotide, d-pTpApCpTpApC. [b] R_f with respect to the trinucleotide, d-TpApC.

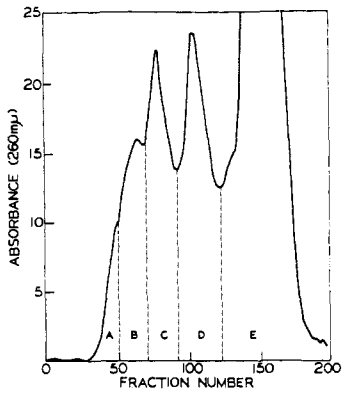

Figure 2. Chromatography on Sephadex G-50 column (90 × 2.5 cm) of the products of polymerization of d-pTpA^{Bz}pG^{Ac} and d-pTpA^{Bz}pG^{Ac}-OAc. Fractions of ~3.5 ml were collected every 15 min.

Polymerization of a Mixture of d-pTpA^{Bz}pG^{Ac} and d-pTpA^{Bz}pG^{Ac}-OAc and the Isolation of Resulting Polynucleotides. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pTpA^{Bz}pG^{Ac} (2500 OD_{280}, 0.07 mmole) and d-pTpA^{Bz}pG^{Ac}-OAc (500 OD_{280}, 0.014 mmole) in dry pyridine (1 ml) was treated with mesitylenesulfonyl chloride (130 mg) under standard conditions for 1.5 hr. After usual work-up the oligonucleotides were separated by Sephadex G-50 column chromatography. The elution pattern is shown in Figure 2 and the distribution of nucleotidic material is given in Table VI.

Table VI. Separation on a Sephadex G-50 Column of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Thymidylyldeoxyadenylyldeoxyguanylate (Elution Pattern in Figure 2)

Peak	Total OD_{260} units	Total eluted,[a] %	Identification
A–C	1300	52.0	Mainly hexa- and higher polynucleotides
D	684	27.3	Mainly tri- and cyclic nucleotides
E	...	...	Nonnucleotidic compounds

[a] Percentage calculated on the basis of 2500 OD_{260} (unprotected nucleotidic material).

For further separation of higher polynucleotides DEAE-cellulose column chromatography (peaks A–C, 1300 OD_{260}) (7 *M* urea–sodium chloride) was used. The results are shown in Table VII.

Table VII. Further Separation on a DEAE-Cellulose (Chloride + 7 *M* Urea) Column of Higher Deoxyribopolynucleotides in Fractions 30–100 from Sephadex G-50 Chromatography (Elution Pattern in Figure 3)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	304–340	202	16.2	Hexanucleotide (54)
B	405–435	75	7.0	Nonanucleotide (43)
C	490–505	9	0.72	Dodecanucleotide (45)
D + imadazole fraction	530–576	84	6.7	Pentadecanucleotide and higher homologs

[a] See footnote *a*, Table IV.

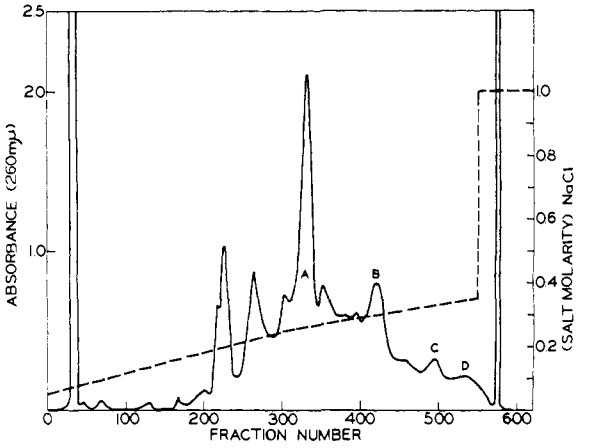

Figure 3. Chromatography of deoxyribopolynucleotides [fractions 30–100 from Sephadex G-50 chromatography (Figure 2)] containing the repeating trinucleotide sequence thymidylyldeoxyadenylyldeoxyguanylate on a DEAE-cellulose (chloride) column (80 × 2 cm) preequilibrated with 0.04 *M* sodium chloride + 7 *M* urea. Elution was carried out with a linear gradient, 1 l. of 0.04 *M* sodium chloride in 7 *M* urea in the mixing vessel, and 1 l. of 0.25 *M* sodium chloride in 7 *M* urea in the reservoir. Fractions of 4 ml were collected every 15 min.

The R_f's and the yields of the homologous products are given in Table VIII.

Polymerization of a Mixture of d-pA^{Bz}pTpC^{An} and d-pA^{Bz}pTpC^{An}-OAc and the Isolation of Resulting Polynucleotides. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pA^{Bz}pTpC^{An} (8000 OD_{280}, 0.22 mmole) and d-pA^{Bz}pTpC^{An} (2000 OD_{280}, 0.044 mmole) in dry pyridine (1 ml) was treated with mesitylenesulfonyl chloride

Table VIII. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Thymidylyldeoxyadenylyldeoxyguanylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group	R_f in solvent C[b] Without 5′-phosphate end group
Cyclo-d-pTpApG	8.0	3.6	3.6	...
d-pTpApG	30.0	2.8	5.3	1.0
d-pTpApGpTpApG	12.0	1.0	2.1	0.40
d-pT(pApGpT)$_2$pApG	2.6	0.33	0.70	0.19
d-pT(pApGpT)$_3$pApG		0.14	0.25	0.10
d-pT(pApGpT)$_4$pApG	9.0		0.11	0.04
Higher homologs				

[a] R_f with respect to the hexanucleotide, d-pTpApGpTpApG. [b] R_f with respect to the trinucleotide, d-TpApG.

(500 mg) under standard conditions for 1.5 hr. After usual work-up the polynucleotides were separated by Sephadex-G-50 column chromatography and paper chromatography. The elution pattern is given in Figure 4 and the distribuion of the nucleotidic material is given in Table IX. The R_f's and the yield of the homologous products are given in Table X.

Polymerization of a Mixture of d-pA^{Bz}pTpG^{Ac} and d-pA^{Bz}pTpG^{Ac}-OAc and the Isolation of Resulting Polynucleotides. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pA^{Bz}pTpG^{Ac} (8000 OD_{280}, 0.21 mmole) and d-pA^{Bz}pTpG^{An}-OAc (2000 OD_{280}, 0.05 mmole) in dry pyridine (1 ml) was treated with mesitylenesulfonyl chloride (500 mg) under standard conditions for 1.5 hr. After usual work-up, the polynucleotides were separated by Sephadex G-50 column chromatography and paper chromatography. The

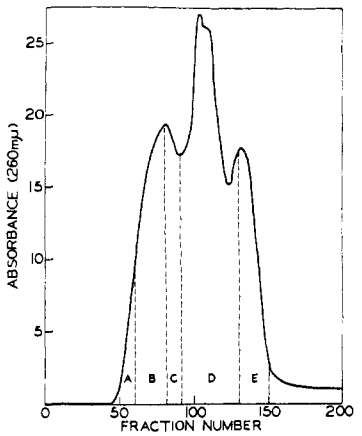

Figure 4. Chromatography on Sephadex G-50 column (90 × 2.5 cm) of the products of polymerization of d-pA^{Bz}pTpC^{An} and d-pA^{Bz}pTpC^{An}-OAc. Fractions of 3.5 ml were collected every 15 min.

elution pattern is shown in Figure 5 and the distribution of the nucleotidic material is given in Table XI. The R_f's and the yields of the homologous products are given in Table XII.

Table IX. Separation on a Sephadex G-50 Column of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxyadenylylthymidylyldeoxycytidylate (Elution Pattern in Figure 4)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	45–60	340	6.4	Dodecanucleotide (20), pentadecanucleotide (15), octadecanucleotide (14), and higher polynucleotides (10)
B	61–80	1580	29.1	Hexanucleotide (27), nonanucleotide (20), and higher polynucleotides (25)
C	81–90	945	17.4	Trinucleotide (21) and hexanucleotide (40)
D	91–130	3655	47.1	Nonnucleotidic material (40), trinucleotide (27), and cyclotrinucleotide (40)

[a] See footnote *a*, Table IV.

Table X. Yield and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxyadenylylthymidylyldeoxycytidylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group	R_f in solvent C[b] With 5′-phosphate end group	R_f in solvent C[b] Without 5′-phosphate end group
Cyclo-d-pApTpC	18.0	3.1	3.1		
d-pApTpC	21.7	2.2	4.4	1.4	1.0
d-pApTpCpApTpC	16.2	1.0	2.1	1.0	0.68
d-pA(pTpCpA)$_2$pTpC	8.6	0.35	0.84	0.55	0.37
d-pA(pTpCpA)$_3$pTpC	3.0	0.15	0.33	0.27	0.24
d-pA(pTpCpA)$_4$pTpC	9.0	0.06	0.13	0.14	0.11
d-pA(pTpCpA)$_5$pTpC		...	...	0.07	0.05
d-pA(pTpCpA)$_6$pTpC		...	...		0.92
Higher Polynucleotides					

[a] R_f with respect to hexanucleotide, d-pApTpCpApTpC. [b] R_f with respect to trinucleotide, d-ApTpC.

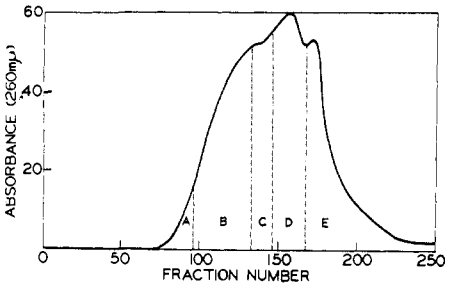

Figure 5. Chromatography on Sephadex G-50 column (90 × 2.5 cm) of the products of polymerization of d-pA^{Bz}pTpG^{Ac} and d-pA^{Bz}pTpG^{Ac}-OAc. Fractions of 3.5 ml were collected every 15 min.

Polymerization of a Mixture of d-pC^{An}pG^{Ac}pT and d-pC^{An}pG^{Ac}pT-OAc and the Isolation of the Resulting Polynucleotides. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pC^{An}pG^{Ac}pT (800 OD_{280}, 0.022 mmole) and d-pC^{An}pG^{Ac}pT-OAc (200 OD_{280}, 0.005 mmole) in dry pyridine (0.5 ml) was treated with mesitylenesulfonyl chloride (60 mg) under standard conditions for 1.5 hr. After usual work-up the polynucleotides were separated by Sephadex G-50 column chromatography and paper chromatography.

Table XI. Separation on a Sephadex G-50 Column of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxyadenylylthymidyldeoxyguanylate (Elution Pattern in Figure 5)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	74–95	450	5.0	Dodecanucleotide (16) and higher polynucleotides
B	96–132	2250	32.5	Hexanucleotide (48) and nonanucleotide (16)
C	133–146	1680	25.0	Trinucleotide (45) and hexanucleotide (18)
D	147–167	3000	22.5	Trinucleotide (50) and nonnucleotidic material (50)
E	168–210	2000	15.0	Cyclotrinucleotide (30), trinucleotide (20), and nonnucleotidic material (50)

[a] See footnote *a*, Table IV.

Table XII. Yield and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxyadenylylthymidylyldeoxyguanylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group	R_f in solvent C[b] Without 5′-phosphate end group
Cyclo-d-pApTpG	9.5	5.0	5.0	...
d-pApTpG	29.0	3.2	6.4	1.0
d-pApTpGpApTpG	20.7	1.0	2.0	0.40
d-pA(pTpGpA)$_2$pTpG	7.5	0.32	0.70	0.18
d-pA(pTpGpA)pTpG	1.5	0.10	0.26	0.10
Higher polynucleotides	5.0			

[a] R_f with respect to hexanucleotide, d-pApTpGpApTpG. [b] R_f with respect to trinucleotide, d-ApTpG.

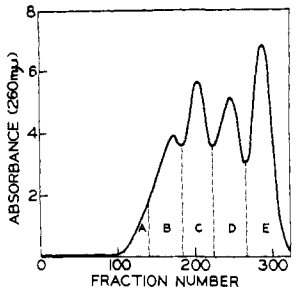

Figure 6. Chromatography on Sephadex G-50 column (90 × 2.5 cm) of the products of polymerization of d-pC^{An}pG^{Ac}pT and d-pC^{An}pG^{Ac}pT-OAc. Fractions of 3.5 ml were collected every 15 min.

The elution pattern is given in Figure 6 and the distribution of the nucleotidic material is given in Table XIII. The R_f's and the yields of the homologous products are given in Table XIV.

Table XIII. Separation on a Sephadex G-50 Column of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxycytidylyldeoxyguanylylthymidylate (Elution Pattern in Figure 6)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	100–140	60.0	8.0	Nonanucleotide (22), dodecanucleotide (2.8), pentadecanucleotide and higher polynucleotides (7)
B	141–184	285.0	51.0	Hexanucleotide (24), nonanucleotide (10), and higher polynucleotides
C	185–224	450.0	41.0	Cyclotrinucleotide (15), trinucleotide (32)
D + E	225–309	904.0	...	Nonnucleotidic material

[a] See footnote *a*, Table IV.

Table XIV. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxycytidylyldeoxyguanylylthymidylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group	R_f in solvent C[b] Without 5′-phosphate end group
Cyclo-d-pCpGpT	12.7	4.0	4.0	...
d-pCpGpT	28.0	3.0	6.0	1.0
d-pCpGpTpCpGpT	14.0	1.0	2.0	0.35
d-pC(pGpTpC)$_2$pGpT	8.4	0.34	0.70	0.19
d-pC(pGpTpC)$_3$pGpT	2.3	0.12	0.26	0.10
d-pC(pGpTpC)$_4$pGpT } Higher polynucleotides	4.0	0.04	0.10	0.05

[a] R_f with respect to hexanucleotide, d-pCpGpTpCpGpT. [b] R_f with respect to trinucleotide, d-CpGpT.

Polymerization of a Mixture of d-pC^{An}pG^{Ac}pA^{Bz} and d-pC^{An}pG^{Ac}pA^{Bz}-OAc and the Isolation of the Resulting Polynucleotides. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pC^{An}pG^{Ac}pA^{Bz} (2000 OD_{280}, 0.04 mmole) and d-pC^{An}pG^{Ac}pA^{Bz}-OAc (500 OD_{280}, 0.01 mmole) in dry pyridine (1 ml) was treated with mesitylenesulfonyl chloride (110 mg) under standard conditions for

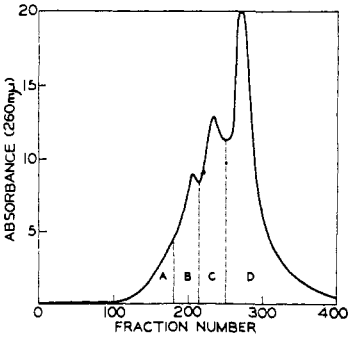

Figure 7. Chromatography on Sephadex G-50 column (90 × 2.5 cm) of the products of polymerization of d-pC^{An}pG^{Ac}pA^{Bz} and d-pC^{An}pG^{Ac}pA^{Bz}-OAc. Fractions of 3.5 ml were collected every 15 min.

1.5 hr. After the usual work-up the polynucleotides were separated by Sephadex G-50 column chromatography and paper chromatograpny. The elution pattern is given in Figure 7 and the distribution of the nucleotidic material is given in Table XV. The R_f's and the yield of the homologous products are given in Table XVI.

Table XV. Separation on a Sephadex G-50 Column of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxycytidylyldeoxyguanylyldeoxyadenylate (Elution Pattern in Figure 7)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	100–180	140	12.5	Nonanucleotide (8), dodecanucleotide (15), and higher polynucleotides
B	181–214	412	37.5	Hexanucleotide (60), nonanucleotide (16)
C	214–250	700	50	Cyclotrinucleotide (30), trinucleotide (50), and nonnucleotidic material (50)
D	251–340			Nonnucleotidic material

[a] See footnote *a*, Table IV.

Table XVI. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxycytidylyldeoxyguanylyldeoxyadenylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group	R_f in solvent C[b] Without 5′-phosphate end group
Cyclo-d-pCpGpA	16.0	4.6	4.6	
d-pCpGpA	27.5	3.3	6.6	1.0
d-pCpGpApCpGpA	21.0	1.0	2.0	0.39
d-pC(pGpApC)$_2$pGpA	8.0	0.33	0.71	0.17
d-pC(pGpApC)$_3$pGpA	1.6	0.13	0.30	0.09
d-pC(pGpApC)$_4$pGpA } Higher polynucleotides	10.0	...	0.13	0.04
		...	...	...

[a] R_f with respect to hexanucleotide, d-pCpGpApCpGpA. [b] R_f with respect to trinucleotide, d-CpGpA.

Polymerization of a Mixture of d-pTpTpG^{Ac} and d-pTpTpG^{Ac}-OAc and the Isolation of the Resulting Polynucleotides. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pTpTpG^{Ac}

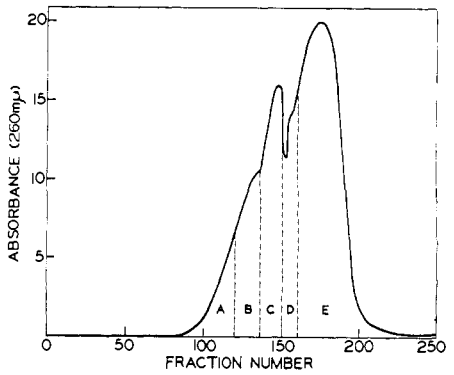

Figure 8. Chromatography on Sephadex G-50 column (90 × 2.5 cm) of the products of polymerization of d-pTpTpG^{Ac} and d-pTpTpG^{Ac}-OAc. Fractions of 3.5 ml were collected every 15 min.

(4500 OD_{280}, 0.14 mmole) and d-pTpTpG^{Ac}-OAc (1000 OD_{280}, 0.021 mmole) in dry pyridine (1 ml) was treated with mesitylenesulfonyl chloride (300 mg) under standard conditions for 1.5 hr. After usual work-up, the polynucleotides were separated by Sephadex G-50 column chromatography and paper chromatography. The elution pattern is shown in Figure 8 and the distribution of the nucleotidic material is given in Table XVII. The R_f's and yields of the homologous products are given in Table XVIII.

Table XVII. Separation on a Sephadex G-50 Column of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Thymidylylthymidylyldeoxyguanylate (Elution Pattern in Figure 8)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	84–120	186.0	6.7	Dodecanucleotide (35), pentadecanucleotide (17), and higher polynucleotides (25)
B	121–136	334.0	12.0	Nonanucleotide (54) and dodecanucleotide (7.5)
C	137–150	700.0	29.0	Hexanucleotide (54) and nonanucleotide (40)
D	151–160	385	14.0	Trinucleotide (50) and hexanucleotide (40)
E	161–200	2530	45.7	Nonnucleotidic material (50), cyclotrinucleotide (27), and trinucleotide (23)

[a] See footnote *a*, Table IV.

Table XVIII. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Thymidylylthymidylyldeoxyguanylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group
Cyclo-d-pTpTpG	13.1	3.4	3.4
d-pTpTpG	19.0	2.4	4.9
d-pTpTpGpTpTpG	25.5	1.0	2.0
d-pT(pTpGpT)$_2$pTpG	7.5	0.35	0.9
d-pT(pTpGpT)$_3$pTpG	2.7	0.15	0.30
d-pT(pTpGpT)$_4$pTpG	1.0	0.05	0.15
Higher Polynucleotides	6.0		

[a] R_f with respect to hexanucleotide, d-pTpTpGpTpTpG.

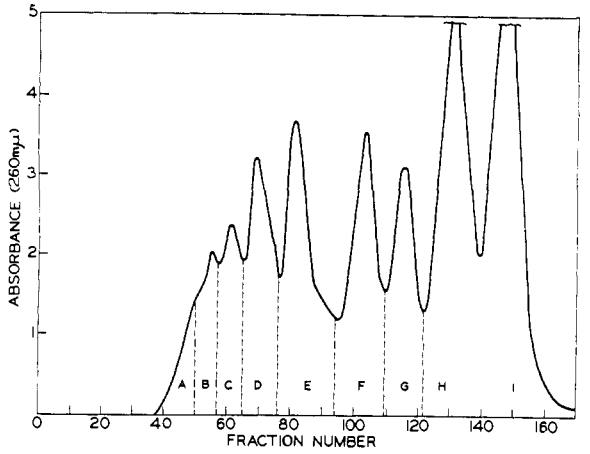

Figure 9. Chromatography on Sephadex G-50 (100 × 2.5 cm) of the products of polymerization of d-pC^{An}pC^{An}pA^{Bz} and d-pC^{An}-pC^{An}pA^{Bz}-OAc. Fractions of ~4 ml were collected every 10 min.

Polymerization of a Mixture of d-pC^{An}pC^{An}pA^{Bz} and d-pC^{An}-pC^{An}pA^{Bz}-OAc and the Isolation of the Resulting Polynucleotides. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pC^{An}-pC^{An}pA^{Bz} (1100 OD_{302}, 0.02 mmole) and d-pC^{An}-pC^{An}-pA^{Bz}-OAc (275 OD_{302}, 0.005 mmole) in dry pyridine (1 ml) was treated with mesitylenesulfonyl chloride (90 mg) under standard conditions for 2 hr. After the usual work-up, the polynucleotides were separated by Sephadex G-50 column chromatography and paper chromatography. The elution pattern is given in Figure 9 and the distribution of the nucleotidic material is given in Table XIX. The yields and R_f's of the homologous products are given in Table XX.

Table XIX. Separation on a Sephadex G-50 Column of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxycytidylyldeoxycytidylyldeoxyadenylate (Elution Pattern in Figure 9)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	38–50	30.4	5.5	Mainly pentadeca- and higher nucleotides and pyrophosphates
B	51–57	45.0	8.2	Pentadecanucleotide (50), dodecanucleotide (10)
C	58–65	60.5	11.0	Dodecanucleotide (60), nonanucleotide (20)
D	66–76	87.7	15.8	Nonanucleotide (80)
E	77–94	127.7	23.1	Hexanucleotide (80)
F	95–110	116.6	21.0	Trinucleotide (80%)
G	111–122	85.2	15.4	Cyclotrinucleotide (80)
H				Mesitylenesulfonic acid
I				Anisic acid and benzoic acid

[a] See footnote *a*, Table IV.

Table XX. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxycytidylyldeoxycytidylyldeoxyadenylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group
Cyclo-d-pCpCpA	12		
d-pCpCpA	17	2.8	5.7
d-pCpCpApCpCpA	18	1	2
d-pC(pCpApC)$_2$pCpA	12	0.3	0.7
d-pC(pCpApC)$_3$pCpA	7.5	0.09	0.2
d-pC(pCpApC)$_4$pCpA	5		

[a] R_f relative to d-pCpCpApCpCpA.

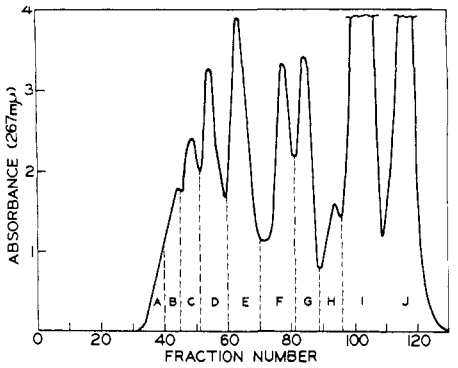

Figure 10. Chromatography on Sephadex G-50 column of the product of polymerization of d-pC^{An}pC^{An}pT and d-pC^{An}pC^{An}pT-OAc. Fractions of ~3.5 ml were collected every 6 min.

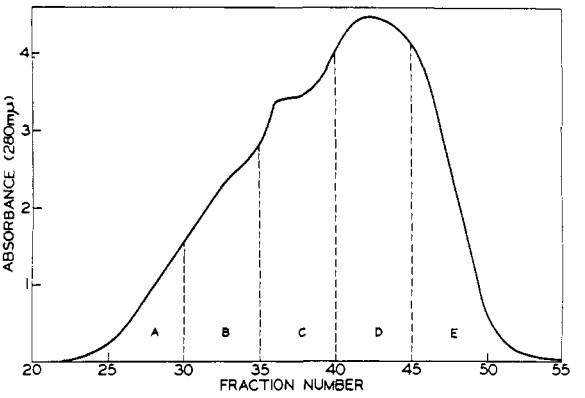

Figure 11. Chromatography on Sephadex G-50 column of the product of polymerization of d-pA^{Bz}pA^{Bz}pG^{Ac} and d-pA^{Bz}pA^{Bz}pG^{Ac}-OAc. Fractions of ~3.5 ml were collected every 6 min.

Polymerization of a Mixture of d-pC^{An}pC^{An}pT and d-pC^{An}pC^{An}pT-OAc. An anhydrous mixture (0.024 mmole) of tri-*n*-hexylammonium salts of d-pC^{An}pC^{An}pT (860 OD_{302}) and d-pC^{An}pC^{An}pT-OAc (215 OD_{302}) in anhydrous pyridine (1 ml) was polymerized under standard conditions using mesitylenesulfonyl chloride (0.088 g, 0.4 mmole). After standard work-up involving treatment with water and triethylamine and then removal of the protecting groups with concentrated ammonia, 90% of the product was concentrated, taken up in 0.1 *M* triethylammonium bicarbonate solution (2 ml), and chromatographed on a Sephadex G-50 (fine) column (2.5 × 100 cm) using 0.1 *M* triethylammonium bicarbonate as the eluent. The elution pattern is given in Figure 10. The distribution of the polymers is given in Table XXI. The R_f's and yields of the polymers are given in Table XXII.

Table XXI. Separation by Sephadex G-50 Column Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxycytidylyldeoxycytidylylthymidylate (Elution Pattern in Figure 10)

Peak	Fraction	Total OD_{270} units	Yield, %	Identification (%[a])
A	34–40	17.5	3.5	Pentadeca- and higher nucleotides and pyrophosphates
B	41–45	34.3	6.8	Pentadecanucleotide (40)
C	46–51	52.9	10.4	Dodecanucleotide (50)
D	52–60	88.0	17.4	Nonanucleotide (60)
E	61–70	102.8	20.3	Hexanucleotide (70)
F	71–81	98.9	19.5	Trinucleotide (75)
G	82–88	68.0	13.5	Cyclotrinucleotide (80)
H	89–96	42.0	8.3	Unidentified
I				Mainly mesitylenesulfonic acid
J				Mainly anisic acid

[a] See footnote *a*, Table IV.

Table XXII. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxycytidylylydeoxycytidylylthymidylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group	R_f in solvent C[b] Without 5′-phosphate end group
Cyclo-d-pCpCpT	10.5			
d-pCpCpT	16	2.6	5.5	
d-pCpCpTpCpCpT	14	1	2	
d-pC(pCpTpC)$_2$pCpT	10	0.35	0.8	
d-pC(pCpTpC)$_3$pCpT	5	0.12	0.27	
d-pC(pCpTpC)$_4$pCpT	3		0.09	

[a] R_f with respect to d-pCpCpTpCpCpT. [b] R_f with respect to d-CpCpT.

Polymerization of a Mixture of d-pA^{Bz}pA^{Bz}pG^{Ac} and d-pA^{Bz}pA^{Bz}pG^{Ac}-OAc. An anhydrous mixture of d-pA^{Bz}pA^{Bz}pG^{Ac} (400 OD_{280}) and d-pA^{Bz}pA^{Bz}pG^{Ac}-OAc (100 OD_{280}) in dry pyridine (2 ml) was treated with triisopropylbenzenesulfonyl chloride (0.16 g). Without further evaporation it was kept at room temperature for 2 hr. Water (1 ml) was added under cooling, and the solution was kept at room temperature for a day after the addition of triethylammonium bicarbonate solution (1 *M*, 5 ml). The solution was evaporated to dryness to remove pyridine and taken up in 0.1 *M* triethylammonium bicarbonate (2.5 ml). The solution was centrifuged and 90% of it was applied to a Sephadex G-75 column (2 × 86 cm) and eluted with 0.1 *M* triethylammonium bicarbonate solution. The elution pattern is given in Figure 11. The distribution of the polymers is given in Table XXIII. The protecting groups were removed after pooling the fractions. The R_f's and yields of the polymers are given in Table XXIV.

Table XXIII. Separation by Sephadex G-75 Column Chromatography of Protected Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence, N-Benzoyldeoxyadenylyl-N-benzoyldeoxyadenylyl-N-acetyldeoxyguanylate (Elution Pattern in Figure 11)

Peak	Fraction	Total OD_{280} units	Total eluted, %	Identification (%[a])
A	22–30	28.2	8.1	Nonanucleotide (10) and higher polymers and pyrophosphates
B	31–35	67.5	19.4	Nonanucleotide (40) and hexanucleotide (40)
C	36–40	94.0	27.0	Hexanucleotide (80)
D	41–45	105.6	30.0	Trinucleotide (80)
E	46–50	53.0	15.2	Trinucleotide (30) and cyclotrinucleotide (60)

[a] Identified after removal of protecting groups. See also footnote *a*, Table IV.

Polymerization of a Mixture of d-pG^{Bz}pG^{Bz}pT and d-pG^{Bz}pG^{Bz}pT-OAc. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pG^{Bz}pG^{Bz}pT (1000 OD_{260} units) and d-pG^{Bz}pG^{Bz}pT-OAc (250 OD_{260} units) in anhydrous pyridine (1 ml) was polymerized under standard conditions (2 hr) using mesitylenesulfonyl chloride (0.88 g). After standard work-up involving treatment with water and triethylamine, the reaction mixture was concentrated to a gum and treated with a 1:1 mixture of methanol and *n*-butylamine (10 ml) for 5 days to remove the protecting groups; 80% of the product was concentrated, taken up in 0.1 *M* triethylammonium bicarbonate solution (2 ml), and chromatographed on Sephadex G-50 (fine) column (2.5 × 100 cm) using 0.1 *M* triethylammonium bicarbonate

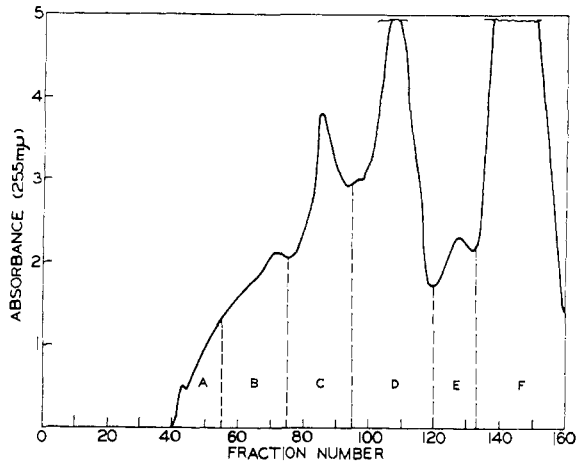

Figure 12. Chromatography on Sephadex G-50 column of the products of polymerization of d-pG^{Bz}pG^{Bz}pT and d-pG^{Bz}pG^{Bz}pT-OAc. Fractions of ~3.5 ml were collected every 6 min.

as the eluent. The elution pattern is given in Figure 12. The distribution of the polymers is given in Table XXV. The R_f values and yields of the polymers are given in Table XXVI.

Table XXIV. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxyadenylyldeoxyadenylyldeoxyguanylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group	R_f in solvent C[b] Without 5′-phosphate end group
Cyclo-d-pApApG	10			
d-pApApG	29	3.2	5.9	1.0
d-pApApGpApApG	30	1.0	1.9	0.68
d-pA(pApGpA)$_2$pApG	8	0.35	0.8	0.45
d-pA(pApGpA)$_3$pApG	3	0.14	0.27	0.29
d-pA(pApGpA)$_4$pApG	1		0.11	0.2

[a] R_f with respect to d-pApApGpApApG. [b] R_f with respect to d-ApApG.

Table XXV. Separation by Sephadex G-50 Column Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxyguanylyldeoxyguanylylthymidylate (Elution Pattern in Figure 12)

Peak	Fraction	Total OD_{255} units	Total eluted, %	Identification (%[a])
A	41–55	39.5	6.1	Dodecanucleotide (10) and higher polymers
B	56–75	122.4	19.0	Nonanucleotide (20) and dodecanucleotide (10)
C	76–95	165.2	25.6	Hexanucleotide (45)
D	96–120	228.2	35.4	Trinucleotide (50)
E	121–133	88.2	13.7	Cyclotrinucleotide (50)
F	134–160			Mainly mesitylenesulfonic acid

[a] See footnote *a*, Table IV.

Polymerization of a Mixture of d-pG^{Bz}pG^{Bz}pA^{Bz} and d-pG^{Bz}pG^{Bz}pA^{Bz}-OAc. An anhydrous mixture of tri-*n*-hexylammonium salts of d-pG^{Bz}pG^{Bz}pA^{Bz} (1728 OD_{260} units) and d-pG^{Bz}pG^{Bz}-

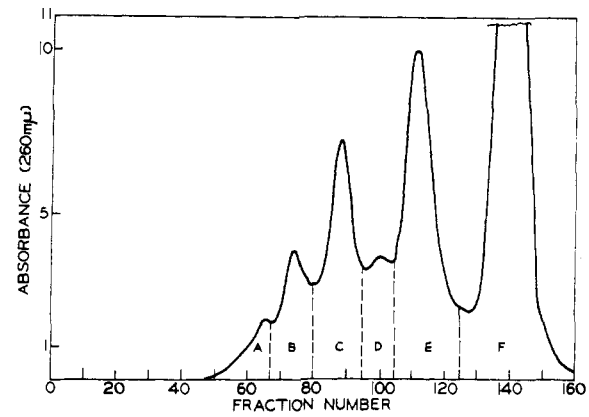

Figure 13. Chromatography on Sephadex G-50 column of the products of polymerization of d-pG^{Bz}pG^{Bz}pA^{Bz} and d-pG^{Bz}pG^{Bz}-pA^{Bz}-OAc. Fractions of ~3.5 ml were collected every 6 min.

Table XXVI. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxyguanylyldeoxyguanylylthymidylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group
Cyclo-d-pGpGpT	7		
d-pGpGpT	18	3.1	6.3
d-pGpGpTpGpGpT	12	1	2
d-pG(pGpTpG)$_2$pGpT	4	0.3	0.65
d-pG(pGpTpG)$_3$pGpT	2.5	0.09	0.2
d-pG(pGpTpG)$_4$pGpT	0.5		0.06

[a] R_f relative to d-pGpGpTpGpGpT.

Table XXVII. Separation by Sephadex G-50 Column Chromatography of Deoxyribopolynucleotide Containing the Repeating Trinucleotide Sequence Deoxyguanylyldeoxyguanylyldeoxyadenylate (Elution Pattern in Figure 13)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	45–67	57.3	6.2	Pentadecanucleotide (10), dodecanucleotide (30)
B	68–80	131.1	14.1	Nonanucleotide (50)
C	81–95	247.5	26.7	Hexanucleotide (50%)
D	96–105	122.5	13.2	Unidentified
E	106–125	368.0	39.7	Trinucleotide (60), cyclotrinucleotide (10)
F	126–160			Mainly mesitylenesulfonic acid

[a] See footnote *a*, Table IV.

Table XXVIII. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Trinucleotide Sequence Deoxyguanylyldeoxyguanylyldeoxyadenylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group
Cyclo-d-pGpGpA	4		
d-pGpGpA	24	3.2	6.5
d-pGpGpApGpGpA	13	1	2
d-pG(pGpApG)$_2$pGpA	7	0.3	0.6
d-pG(pGpApG)$_3$pGpA	1.5	0.09	0.2
d-pG(pGpApG)$_4$pGpA	0.5		0.06

[a] See footnote *a*, Table IV.

pA^{Bz}-OAc (432 OD_{260} units) in anhydrous pyridine (1 ml) was polymerized under standard conditions (2 hr) using mesitylenesulfonyl chloride (0.15 g). After the standard work-up involving treatment with water and triethylamine, the reaction mixture was concentrated to a gum and treated with a 1:1 mixture of methanol and *n*-butylamine (10 ml) for 5 days to remove the protecting groups; 80% of the product was concentrated, taken up in 0.1 *M* triethylammonium bicarbonate solution (2 ml) and chromatographed on a Sephadex G-50 (fine) column (2.5 × 300 cm) using 0.1 *M* triethylammonium bicarbonate as the eluent. The elution pattern is given in Figure 13. The distribution of the polymers is given in Table XXVII. The R_f values and yields of the polymers are given in Table XXVIII.

Acknowledgment. The technical assistance of Harry Radloff is acknowledged with appreciation.

Studies on Polynucleotides. LXIV.[1] The Synthesis of Deoxyribopolynucleotides Containing Repeating Tetranucleotide Sequences (1)[2]

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Contribution from the Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin 53706. *Received December 12, 1966*

Abstract: Syntheses of three homologous series of deoxyribopolynucleotides containing the following repeating tetranucleotide sequences are described: (1) thymidylylthymidylylthymidylyldeoxycytidylate, (2) deoxyadenylyldeoxyadenylyldeoxyadenylyldeoxyguanylate, and (3) deoxyadenylylthymidylyldeoxycytidylyldeoxyguanylate. The synthesis of the first series was accomplished by the stepwise method which involved repetitive condensation of a suitably protected mononucleotide to the 3′-hydroxyl end group of a growing oligonucleotide chain. Syntheses of deoxyribopolynucleotides belonging to the other two series were accomplished by the polymerization of suitably protected tetranucleotides.

The usefulness of ribopolynucleotides with repeating tetranucleotide sequences in studies of the genetic code has been reviewed in an accompanying paper.[3] As with ribopolynucleotides containing repeating di- and trinucleotide sequences, the first requirement in the preparation of ribopolynucleotides with repeating tetranucleotide sequences was the preparation of short-chain deoxyribopolynucleotides containing the appropriate repeating nucleotide sequences. The work described in this paper formed the first phase of the total synthetic work dealing with the preparation of deoxyribonucleotides containing repeating tetranucleotide sequences. Syntheses of the following three series of deoxyribopolynucleotides have been accomplished: (1) a dodecanucleotide containing the repeating thymidylylthymidylylthymidylyldeoxycytidylate sequence, (2) octa- and dodecadeoxyribonucleotides containing the repeating deoxyadenylyldeoxyadenylyldeoxyadenylyldeoxyguanylate sequence, and (3) octa-, dodeca-, and hexadecanucleotides containing all four mononucleotides in the repeating deoxyadenylylthymidylyldeoxycytidylyldeoxyguanylate sequence. Two accompanying papers[4,5] describe continuation of the work dealing with the synthesis of deoxyribopolynucleotides containing repeating tetranucleotide sequences.

The choice of the repeating tetranucleotide sequences synthesized in this paper was determined by the following considerations. At the start of the present work, DNA-dependent RNA polymerase was the only enzyme which had been used successfully in preparation of a long ribopolynucleotide with a repeating trinucleotide sequence.[6] Thus the use of the previously synthesized nonanucleotide containing the repeating thymidylylthymidylyldeoxycytidylate sequence as template for this enzyme had afforded a long ribopolynucleotide containing the complementary ribonucleotides in repeating sequence. In contrast, attempts to use several other series of short deoxyribopolynucleotides containing purine nucleotides as templates for the above enzyme had given negative results.[7] In extending the use of DNA-dependent RNA polymerase to the preparation of long ribopolynucleotides containing repeating tetranucleotide sequences, it was therefore concluded that deoxyribopolynucleotides containing only pyrimidine nucleotide units in them had the maximum chance of serving as templates. The synthesis of the deoxyribopolynucleotide containing the repeating tetranucleotide sequence thymidylylthymidylylthymidylyldeoxycytidylate sequence was therefore undertaken. Further considerations in favor of this particular sequence were that it required the minimum of protecting groups and that the methodology for chromatography and isolation at successive synthetic steps had been satisfactorily worked

(1) Preceding paper in this series: S. A. Narang, T. M. Jacob, and H. G. Khorana, *J. Am. Chem. Soc.*, **89**, 2167 (1967).

(2) This work has been supported by grants from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service (Grant No. CA-05178), the National Science Foundation (Grant No. GB-3342), and the Life Insurance Medical Research Fund (Grant No. G-62-54).

(3) H. G. Khorana, H. Büchi, T. M. Jacob, H. Kössel, S. A. Narang, and E. Ohtsuka, *J. Am. Chem. Soc.*, **89**, 2154 (1967).

(4) H. Kössel, H. Büchi, and H. G. Khorana, *ibid.*, **89**, 2185 (1967).

(5) E. Ohtsuka and H. G. Khorana, *ibid.*, **89**, 2195 (1967).

(6) S. Nishimura, T. M. Jacob, and H. G. Khorana, *Proc. Natl. Acad. Sci. U. S.*, **52**, 1494 (1964).

(7) Thus, for example, short-chain deoxyribopolynucleotides containing alternating deoxyadenylate and deoxyguanylate units and another series containing alternating thymidylate and deoxyguanylate units had failed to serve as templates for RNA polymerase: unpublished results of Drs. S. Nishimura and B. D. Mehrotra.