the effect being principally on cells forming 19*S* antibody and only to a slight extent on 7*S*-forming cells (*10*). In preliminary tests with rabbits, one intravenous administration of polyA + polyU produced an elevated titer of circulating antibodies to sheep red blood cells at 7, 12, and 18 days after immunization when the antigen dosage was low (10^8 cells intravenously); no elevation was detected when the antigen dosage was higher (10^9 cells intravenously).

Kinetin riboside (KR) abolishes the stimulatory effects of oligodeoxyribonucleotides and of certain adjuvants on antibody-forming cells (*1*). This compound and also another recently isolated adenosine derivative, N^6-(Δ^2-isopentenyl)adenosine which is an integral part of the soluble RNA of yeast and mammalian tissue (*11*), causes a significant reduction, frequently a total elimination, of the stimulation produced by polyA + polyU (Tables 1 and 2). The unstimulated, normal response is not affected by KR or N^6-(Δ^2-isopentenyl)adenosine. These results suggest (i) that the stimulatory effects produced by certain adjuvants, by oligodeoxyribonucleotides and by polyA + polyU (all reversible by KR) result from an alteration of identical, or at least very similar, biosynthetic events (*8*), and (ii) that the KR-susceptible cells participating in the stimulated primary response may be different from those that are involved in the normal, nonenhanced response (which is unaffected by KR). The latter possibility and the possible role of nucleotide kinases in the stimulation of antibody-forming cells are now under study.

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Deoxycytidylate and Deoxyguanylate Kinase Activity in Pneumococci after Exposure to Known Polyribonucleotides

Abstract. *Polycytidylic acid and to a lesser extent polyadenylic acid enhance the activity of deoxycytidylate and deoxyguanylate kinases in resting cell suspensions of encapsulated pneumococci. The active intracellular materials appear to be oligomers of A and C, respectively. The stimulation of the kinase activities is amino-acid dependent and can be abolished by the addition of chloramphenicol. The addition of all eight naturally occurring deoxyribonucleosides and deoxyribonucleotides to cell suspensions containing the homopolymers leads to a selective enhancement of DNA synthesis.*

Mixtures of oligodeoxyribonucleotides (derived from various deoxyribonuclease-treated DNA's) exert stimulatory effects on rates of DNA synthesis in resting-cell suspensions of virulent pneumococci and streptococci provided that all eight of the naturally occurring deoxyribonucleosides and -tides are present (*1*). This stimulation was related to the ability of oligodeoxyribonucleotides to increase the amounts of all four deoxyribonucleoside triphosphates required for DNA synthesis by increasing the activity of two of the four specific kinases involved in synthesizing the triphosphates. The kinases affected, dCMP (*2*) and dGMP kinases, are present in very low amounts initially in unsupplemented cell suspensions. The enhancement was dependent on amino acids and could be inhibited by the addition of chloramphenicol (*1*). Ribonucleic acid digests (prepared by the exposure of a number of RNA's to pancreatic ribonuclease), and other RNA derivatives were incapable of affecting DNA synthesis of pneumococci. However, subsequent studies by Braun (*3*) with antibody-forming mammalian cells which also are stimulated by oligodeoxyribonucleotides revealed that comparable stimulations can be produced by a molecular complex between homopolymers of adenylic and uridylic acids (polyA + polyU). Polyadenylic acid proved to be the active polyribonucleotide. It was therefore of interest to determine whether such complexes and other homopolymers would affect deoxyribonucleotide kinase activity and DNA synthesis in resting-cell suspensions of pneumococci. A strain pneumococcus of type III (A66) which responds maximally to the oligodeoxyribonucleotides was used. Methods for extracting, partially purifying, and assaying deoxyribonucleotide kinases as well as measuring nucleic acid and protein syntheses in cell suspensions were those used earlier (*1*).

The kinase assays (Table 1) showed that polyC (*4*) increases the activity of both dCMP and dGMP kinases and that low concentrations of polyA enhance dGMP kinase activity. Both polyU and polyI were ineffective, and so were molecular complexes between (polyC + polyI) and (polyA + polyU). These results have been replicated consistently under the environmental conditions used (*1*). Treatment of polyC with concentrations of pancreatic ribonuclease that caused extensive degradation of polyC to CMP, resulted in a loss of kinase enhancement; however, treatment of polyC with *small* amounts of ribonuclease produced greater stimulatory effects on dCMP and dGMP kinase activity than polyC alone. This result, suggesting that oligomers of cytosine were more active than the homopolymer, was supported by the finding that the addition of C^{14}-polyC to cell suspensions resulted, after incubation, in the recovery of most of the cell-associated radioactivity in the fraction soluble in cold trichloroacetic acid rather than in the fraction soluble in hot trichloroacetic acid nucleic (*5*). Paper chromatography of the acid-soluble extract in a solvent consisting of propanol, NH_4OH, and H_2O (55·10·35, by volume) for 60 hours on Whatman No. 3 paper (*6*) showed that 77 percent of this extract was in the form of C^{14} oligomers of cytosine while the remainder was C^{14}-CMP. The radioactivity that was found in the nucleic acid

Table 1. Effects of homopolymers of ribonucleotides on deoxycytidylate and deoxyguanylate kinase activities in pneumococci. Methods for incubation of cells and procedure for extracting and assaying kinases have been described (*1*). Prior to enzyme extraction, the cells were incubated for 25 minutes at 37°C. Specific activity is defined as the number of millimicromoles of C^{14}-product formed per hour per milligram of protein. Protein was determined by the method of Lowry *et al.* (*10*). Concentrations of homopolymers were as follows (micrograms per milliliter in final suspending medium): polyC, 112; polyA, 25; polyU, 25 or 112; polyI, 25 or 112; polyA + polyU, 12.5 each; polyC + polyI, 56 each. The concentrations chosen were those which stimulated kinase activity to its maximum extent under conditions used (*1*).

Additions	Kinase specific activity	
	dCMP	dGMP
polyA	4.0	9.3
polyC	17.5	18.3
polyU	3.0	3.1
polyI	2.9	4.9
polyA + polyU	3.8	5.4
polyC + polyI	4.2	5.0
none	3.5	3.2

extract proved to be both undergraded polyC and cellular C^{14}-RNA from incorporated C^{14}-CMP (*7*).

The enhancement of dCMP and dGMP kinase activity by oligomers of cytosine was dependent on an external source of amino acids and was inhibited by the addition of chloramphenicol, suggesting that, as in the case of stimulation by oligodeoxyribonucleotides (*1*), protein synthesis, rather than activity, is involved in the enhancement. The homopolymers also do not affect kinase activity when added to isolated enzyme extracts. Analysis of the oligomer effects on DNA synthesis in cell suspensions of pneumococci in the presence of a substrate pool of deoxyribonucleosides and deoxyribonucleotides revealed that a selective enhancement of DNA synthesis occurred in comparison to RNA and protein synthesis (Table 2) in a manner similar to that observed with oligodeoxyribonucleotides (*1*).

Table 2. Effects of polyC, polyA, or both, on macromolecular syntheses in pneumococci maintained in the presence of deoxyribonucleosides and deoxyribonucleotides. Methods for extraction and assay of nucleic acids and protein have been described (*1*). Concentrations of supplements were as follows (micrograms per milliliter in medium): polyC, 112; polyA, 25; polyC + polyA, 112 + 25; deoxyribonucleosides, 800 (200 μg each of deoxyadenosine, deoxyguanosine, thymidine, and deoxycytidine); deoxyribonucleotides, 800 (200 μg each of the phosphorylated derivatives described above). S + T indicates deoxyribonucleosides plus deoxyribonucleotides.

Additions			Increase after 70 minutes (%)		
polyC	polyA	(S + T)	DNA	RNA	Protein
+	−	+	85	14	15
−	+	+	53	14	19
+	+	+	91	17	17
−	−	+	35	20	20
+	−	−	18	15	14
−	+	−	20	20	19
+	+	−	23	15	19
−	−	−	20	16	18

All of the above results suggest that oligoribonucleotides of cytosine (and probably of adenine) and oligodeoxyribonucleotides derived from the enzymatic digestion of DNA affect the same biosynthetic processes. As to the observation that pancreatic ribonuclease digests of RNA are ineffective in the bacterial system (*1*) and in the mammalian cell system (*3*), it may be suggested that such RNA digests are deficient in sequences of cytosine because of the specificity of pancreatic ribonuclease in hydrolyzing only pyrimidine-3′-phosphate esters in RNA (*8*). In contrast, pancreatic deoxyribonuclease hydrolyzes phosphodiester bonds between a purine and pyrimidine in DNA (*9*), thus insuring that sequences of cytosine in the oligomer will be maintained. It is also likely that cytosine and adenine oligomers affect the same biosynthetic processes in the bacterial system and in the mammalian cell system (*3*) despite the differences in requirement for single homopolymers and double-stranded molecular complexes, respectively. These differences may merely reflect a rapid degradation of homopolymers in the animal's circulation, thus necessitating protection from nuclease action through the injection of active material in the form of a complex. Once such a complex is incorporated into the cell, it could be degraded over a period of time to active oligomers. In the bacterial system, nuclease activity may not be as pronounced in the extracellular environment, and single-stranded homopolymers can remain relatively intact until they are incorporated by the cells and degraded to active oligomers by intracellular nucleases. A molecular complex might be more difficult to degrade by bacteria during the short time (25 minutes) of the assay period, and consequently little or no active oligomers would become available.

The actual mechanism leading to the enhancement of kinases remains unknown. It may involve derepression, conceivably through interaction between repressor and the oligonucleotide or between repressor-target and oligonucleotide. Efforts are under way to elucidate such possibilities through a study of complex formation between oligo-C and pneumococcal RNA or DNA.

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5. After C^{14}-polyC was added to the bacterial culture and the bacteria were incubated for 25 minutes at 37°C, the cultures were centrifuged and washed four times with cold sodium-potassium phosphate buffer (0.02*M*, *p*H 7.5). The bacteria were then suspended in 5 ml of buffer and a portion was taken for determination of radioactivity. To the remaining suspension 40 percent trichloroacetic acid was added to yield 10 percent by volume, the cells were centrifuged, and the precipitate was washed three times with 3 ml of cold 10 percent trichloroacetic acid. The supernatant was saved for radioactivity determination, and the precipitate was washed twice with 3 ml of 95 percent ethanol and once with 4 ml of a mixture of ethanol and ether (3 : 1) at 60°C for 15 minutes. Two milliliters of 5 percent trichloroacetic acid were added to the precipitate and the nucleic acids were extracted by heating for 30 minutes at 90° to 95°C. The extract was centrifuged and the supernatant was saved for determination of radioactivity (Nuclear-Chicago windowless flow counter and scaler). Specific activity was defined as the number of counts per minute per unit volume of cell suspension.
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