Physical studies have thus far failed to show conclusively whether one or both strands of native donor DNA are physically integrated into the *Bacillus subtilis* recipient genome (*15*). The fact that only one donor strand is genetically effective in a given cell (*13*, *14*) argues against double-strand integration; but it does not rule out the possibility that two strands are integrated and one is enzymatically repaired to match the other. This double-strand integration model is, however, difficult to reconcile with the finding that either strand can be the genetically effective one. The putative repair enzyme would have to correct exclusively the H strand in one cell and exclusively the L strand in another over a region of several genes (*13*). The need for this unlikely repair specificity makes the double-strand integration model less attractive. A single-strand integration model is more consonant with the findings reported here, which would then imply that either strand can be integrated.

Both complementary DNA strands have been found effective in transformation of *Diplococcus pneumoniae* and *Hemophilus influenzae* (*16*) as well as of *Bacillus subtilis*. All three likewise have been found susceptible to transformation by single-stranded DNA (*5*, *7*, *17*). Physical studies have shown single-strand integration in *D. pneumoniae* and *H. influenzae* (*18*), and segregation studies suggest the same mechanism for *B. subtilis* (*13*, *14*). It thus appears that there are extensive similarities underlying the seeming diversity of these three transformation systems.

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Antibody Formation: Stimulation by Polyadenylic and Polycytidylic Acids

Abstract. *Complexes of polyadenylic and polyuridylic acids, or of polycytidylic acid and methylated bovine serum albumin, enhance the early rate of increase in numbers of antibody-forming spleen cells in mice immunized with sheep red blood cells or other particulate antigens. Polyadenylic and polycytidylic acids, respectively, appear to be the source of the critical stimulators which, as demonstrated by others in bacteria, may act by influencing nucleotide kinase activity. The stimulated antibody response, but not the normal response, is antagonized by kinetin riboside and by an adenosine derivative occurring in sRNA.*

Oligodeoxyribonucleotides, such as those obtained after digestion of calf-thymus DNA by pancreatic deoxyribonuclease, enhance the early rate of increase in numbers of antibody-forming spleen cells (*1*), as determined after immunization of mice with sheep red blood cells. The active oligomers also enhance cell multiplication and rates of DNA synthesis in Gram-positive bacteria (*2*, *3*). In bacteria, these stimulatory effects are produced by di- to hexanucleotides (*4*), and tests with pneumococci have indicated that the effects on DNA synthesis are the consequence of stimulated nucleotide kinase activity (*3*). In studies on antibody-forming cell populations of mice, we have observed that stimulatory effects, equal to those observed after oligodeoxyribonucleotide administration, can also be obtained with the aid of homopolymers of certain ribonucleotides. Thus, homopolymers of adenylic acid (polyA) or cytidylic acid (polyC) injected in appropriate form—simultaneously with, shortly before, or shortly after—the injection of antigen enhance antibody formation. Firshein subsequently demonstrated that these homopolymers also stimulate DNA synthesis and nucleotide kinase activity in pneumococci (*5*).

CF-1 or AKR mice, weighing approximately 20 g, were injected in the tail vein with 10^8 or 10^9 sheep erythrocytes per animal; at the same time, polyA, polyC, or polyU (polyuridylic acid) was injected intravenously in quantities ranging from 100 to 1000 μg per animal. Forty-eight hours later, the animals were killed, and the number of spleen cells forming or releasing antibody to sheep red blood cells was determined (*6*); groups of five animals each were used in all of these assays. No stimulated responses were obtained when any of the three homopolymers were injected at time of antigen administration. However, a mixture of all three (750 μg each of polyA, polyC, and polyU per mouse) produced a significant increase in the number of hemolysin-forming spleen cells (per 10^8 spleen cells) 48 hours later (an average of 854.4 in contrast to 246.8 in the controls receiving antigen only). Testing of the homopolymers in pairs revealed that the combined administration of polyA and polyU was responsible for the stimulation (Table 1). When polyA and polyU were injected separately into different sites (one intraperitoneally and the other intravenously) no stimulation occurred; a degree of stimulation did occur when they were administered consecutively into the same site at 30-minute intervals, but not at 3-hour intervals (Table 1). These observations suggested that a double-stranded polymer of polyA and polyU, readily

Table 1. Influence of polyA + polyU on the average number (± S.E.) of hemolysin-producing spleen cells (HP) in CF-1 mice 48 hours after intravenous immunization with sheep red blood cells.

Treatment of spleen donors		HP per 10^8 nucleated spleen cells after 48 hours (Av. No. ± S.E.)
Sheep cells (No.)	Supplement	
	Experiment 1	
None		15.6 ± 11.6
10^8		244.8 ± 29.6
10^8	polyA + polyU*	992.0 ± 216.1
10^8	polyA†	210.6 ± 39.6
10^8	polyU†	192.8 ± 50.4
10^8	polyA + polyU* + KR‡	397.0 ± 78.1
	Experiment 2	
10^8		247.4 ± 41.2
10^8	polyA + polyU*	1064.2 ± 188.8
10^8	polyA i.p. + polyU i.v.§	325.2 ± 33.3
10^8	polyA i.v. + polyU i.v. 30 min later	818.0 ± 79.0
	Experiment 3	
None		28.0 ± 5.1
10^8		326.0 ± 37.6
10^8	polyA + polyU ‖	911.6 ± 78.6
10^6		63.4 ± 19.2
10^6	polyA + polyU ‖	255.2 ± 48.2

* 750 μg per mouse of each, partially intravenously and partially intraperitoneally. † 1500 μg per mouse of each, partially intravenously and partially intraperitoneally. ‡ Kinetin riboside, 2 mg per mouse intraperitoneally. § 750 μg per mouse of each. ‖ 150 μg per mouse of each intravenously.

formed after mixing of polyA and polyU, may be required for the initiation of stimulatory effects. Since such double-stranded polymers have increased resistance to enzymatic degradation, other means for protecting polyA or polyU against ribonucleases in vivo were tested. We knew that methylated bovine serum albumin (MBSA) forms complexes with polynucleotides and that the resulting complex displays increased resistance to enzymatic degradation (*7*). Therefore, polyA and polyU, respectively, were mixed with MBSA, and the resulting complexes were injected with sheep red blood cells into mice. The complex of polyA and MBSA stimulated (Table 2), although not to the same extent as the double-stranded polyAU polymer. The polyU–MBSA complexes yielded equivocal results; one lot of polyU led to stimulation, another lot failed to do so.

In that polyG was unavailable, a complex of polyC and MBSA was tested and found to have some activity (Table 2). These findings support the belief that single-stranded A (adenosine) and C (cytidine) sequences, respectively [and possibly also U (uridine) sequences], may be responsible for the stimulation, but that complex formation of the homopolymer with a basic protein or with a complementary homopolymer is needed to protect active oligomers from rapid enzymatic degradation. This conclusion is strengthened by the demonstration that polyA and polyC, as noncomplexed homopolymers, stimulate a presumably related bacterial system (*8*), that is, DNA synthesis in pneumococci (*5*). The differences in the manner in which the polymers must be used in the mammalian as contrasted with the bacterial test system may merely reflect differences in nuclease activities between mice and pneumococci.

Several sets of data suggest that the biochemical events involved in oligodeoxyribonucleotide and oligonucleotide effects on bacterial DNA synthesis and growth may be similar to those involved in stimulated antibody formation, namely, an enhancement of nucleotide kinases (*8*). This suggestion is further supported by the finding (Table 2) that the response to sheep red blood cells in mice (in terms of number of antibody-forming cells after 48 hours) also is stimulated when antigen is administered together with a mixture of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and thymidine triphosphate (dATP, dCTP, dGTP, and TTP) (150 μg/mouse of each). The resulting enhancement is about equal to that obtained with polyA + polyU if numbers of plaques per one-fifth spleen are compared (sheep red blood cells, 63.4; sheep red blood cells + polyA + polyU, 175.3; sheep red blood cells + deoxynucleoside triphosphates, 150.6); but it is less in terms of plaques per 10^8 spleen cells (Table 2) in that the supplementation with triphosphates increases the total number of cells per spleen. No enhancement is obtained when any one of the four deoxynucleoside triphosphates is omitted, and no enhancement occurs when a mixture of all four deoxynucleoside monophosphates is used. As to the problem of uptake of such materials by mammalian cells, effects of extra-cellular nucleoside monophosphates and triphosphates on intracellular reactions have been reported (*9*).

Dose response tests with polyA + polyU showed that a significantly higher number of specific antibody-forming spleen cells can be obtained 48 hours after antigen administration if as little as 40 μg of each homopolymer is

Table 2. Influence of complexed and uncomplexed homopolymers, and of deoxynucleoside triphosphates, on the number of hemolysin-producing spleen cells (HP) in mice 48 hours after immunization with 10^8 sheep red blood cells.

Treatment of spleen donors		HP per 10^8 nucleated spleen cells after 48 hours (Av. No. ± S.E.)
Sheep cells	Supplement	
	AKR mice	
−	None	29.8 ± 8.7
+	None	466.0 ± 43.6
+	polyA + polyU*	2287.6 ± 209.4
+	polyA†	530.0 ± 52.2
+	polyA + MBSA*	1115.0 ± 127.7
+	MBSA†	437.0 ± 20.7
	CF-1 mice	
−	None	33.4 ± 31.7
+	None	392.6 ± 71.0
+	polyC‡	325.2 + 84.8
+	polyC + MBSA*	694.6 ± 100.5
+	MBSA‡	230.2 + 51.3
	CF-1 mice	
−	None	17.3 ± 10.6
+	None	282.8 ± 87.5
+	polyA + polyU*	794.7 ± 108.7
+	dATP, dGTP, dCTP, TTP*	479.8 ± 52.2
+	dATP, dGTP, dCTP, TTP* + KR	242.6 ± 55.8

* 150 μg per mouse of each intravenously. † 150 μg per mouse intravenously. ‡ 300 μg per mouse intravenously.

administered; even 20 μg of each produces some stimulation. Exposure to ribonuclease (enzyme-substrate ratio 1:100) abolishes the effects of polyA + polyU; however, AAAA (tetraadenylic acid), particularly in combination with polyU, stimulates significantly; AA (diadenylic acid) stimulates to some extent.

The administration of polyA + polyU (150 μg of each per animal were used routinely in all subsequent tests) also enhances responses to antigens other than sheep red blood cells, such as chicken red blood cells or *Escherichia coli*. The cytokinetics of the stimulated response was analyzed in detail in tests with sheep red blood cells. Stimulation was most pronounced during the first 48 hours after antigen administration and was still discernible as late as 96 hours after antigen. However, 120 or 144 hours after antigen administration the number of antibody-forming spleen cells was about the same in groups that received antigen alone, compared to groups that had been exposed to the mixture of antigen, and polyA + polyU. The relative extent of stimulation is greater when low doses of antigen are used compared to higher doses (Table 1). The secondary response also is slightly stimulated by polyA + polyU,

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