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Biologic Properties of Polynucleotides. IV. Studies on the Mechanism of Complement Inhibition by Polyinosinic Acid Together with Observations on the *In Vivo* Effect of Polyinosinic Acid on Complement Activity *

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In recent reports from this laboratory certain synthetic homoribopolynucleotides have been found to possess anti-C' [1] activity (1, 2); of these, poly I is the most potent, as little as 0.0076 μmole P of this material being capable of inhibiting one $C'H_{50}$ (1). Attempts to define the mechanism of action of poly I by use of the R reagents were inconclusive, but suggested that C'1 might be most vulnerable to inactivation by poly I (1). In order to elucidate the mechanism of C' inhibition by poly I, experiments utilizing the kinetic analysis methods of Mayer and others were designed (3). The results of these experiments, with which the present report is concerned, indicate that poly I does in fact selectively inhibit C'1. In contrast to other potent C'1 inhibitors such as DFP (4) and the C'1 esterase serum inhibitor (5), poly I has no effect on C'1 esterase activity. In addition, poly I has been found to be an effective *in vivo* inhibitor of C' activity when administered to Wistar rats.

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[1] The following abbreviations are used: C', complement; anti-C', anticomplementary; $C'H_{50}$, 50% hemolytic unit of complement; C'1, C'2, C'3, C'4, the first, second, third, or fourth component of complement; R1, R2, R3, R4, serum lacking the designated component of complement; EA, sensitized sheep erythrocytes; EAC' . . ., per-sensitized erythrocytes bearing complement components as designated by numerical subscript; E*, red cells irreversibly damaged by the action of complement; DFP, diisopropylfluophosphate; poly I, polyinosinic acid; EDTA, ethylenediamine tetraacetate; ALTEE, *N*-acetyl-L-tyrosine ethyl ester.

Methods

Polyinosinic acid. The synthesis of this material was as previously described (1).

Guinea pig serum. Guinea pig blood obtained by cardiac puncture was allowed to clot for 4 hours at room temperature. The serum was then separated and stored in samples at −40° C.

Salts of EDTA.[2] Na_2H_2 EDTA was prepared in a stock solution of 0.15 M and titrated to pH 7.4 (at this pH it is largely in the form Na_3HEDTA). Na_2Mg EDTA solutions were prepared in an identical manner.

Buffer diluents. Barbital-buffered saline (BBS), pH 7.4, containing 1.5×10^{-4} M Ca^{++} and 5×10^{-4} M Mg^{++} was employed as a diluent in most experiments. BBS–Na_3HEDTA buffer contained 7.5×10^{-3} M Na_3HEDTA; BBS–Na_2Mg EDTA buffer contained 15×10^{3} M Na_2Mg EDTA. BBS containing 1×10^{3} M Ca^{++} only was used in the preparation of C'y.

Preparation of EA. The procedure used in sensitizing sheep red cells has been described previously (1). The usual concentration of EA was 5×10^{8} per ml; if more concentrated cell preparations were desired, the cells were spun down and resuspended in a smaller volume. In performing experiments with R1 it was necessary to sensitize the red cells in Na_3HEDTA-BBS, and subsequently wash them 3 times, once in Na_3HEDTA–BBS and twice in BBS, in order to avoid attachment of the C'1 present in commercial glycerinated hemolysin (6). Failure to do this resulted in high blank lysis of EA in R1.

Preparation of guinea pig C' reagents. R reagents were prepared from freshly drawn guinea pig serum by the procedures outlined in Kabat and Mayer's text (7). The R3, prepared by incubating guinea pig serum with 13.0 mg zymosan [3] per ml at 37° C for 90 minutes, proved

[2] Geigy Industrial Chemicals, Ardsley, N. Y.

[3] Lot no. 2F 1581, Standard Brands, New York, N. Y.

TABLE I

*The sequence of events occurring during immune hemolysis**

EA + C'1q + C'1r + C'1s $\xrightarrow{Ca^{++}}$	EAC'_1
EAC'_1 + C'4 $\longrightarrow$	$EAC'_{1,4}$
$EAC'_{1,4}$ + C'2 $\xrightarrow{Mg^{++}}$	$EAC'_{1,4,2}$
$EAC'_{1,4,2}$ + C'3a + C'3b + C'3c $\rightarrow$	E*
E* $\longrightarrow$	ghost + hemoglobin

* See text (footnote 1) for abbreviations.

to be an excellent reagent for the preparation of $EAC'_{1,4,2}$. C'x (guinea pig serum depleted of Ca^{++} and Mg^{++} by IRC 50 resin treatment) and C'y (a reagent containing only C'2 and C'3) were prepared according to the technique described by Levine and Mayer (8).

Preparation of complexes between C' and EA (persensitized cells). General considerations. Although recent work indicates that C'1 and C'3 are complex factors each consisting of at least three separate components (9, 10), the basic concepts regarding the sequence of action of C' components in the lysis of EA remain unchanged (7*a*). In the presence of Ca^{++}, EA reacts with C'1 to form EAC'_1; at the same time the esterase associated with C'1 is activated. C'1 esterase interacts with C'4 to yield $EAC'_{1,4}$. The latter complex, in the presence of Mg^{++}, reacts with C'2 to form $EAC'_{1,4,2}$; this step also requires the presence of C'1 and C'1 esterase activity, but C'1 is not required for the subsequent stages of immune red cell lysis. $EAC'_{1,4,2}$, even in the absence of divalent cations, then react with C'3 to form $EAC'_{1,4,3,2}$ or E*. E*, an irreversibly damaged cell, loses its hemoglobin and becomes a ghost. This sequence of events is schematically outlined in Table I.

$EAC'_{1,4,2}$. This intermediate complex was prepared with R3 in the following manner: 10 ml EA (5×10^8 per ml) and 0.5 ml R3 were mixed together at 1° C for 30 minutes. The cells were then washed 3 times with cold BBS and resuspended at a concentration of 5×10^8 per ml in cold Na_3HEDTA–BBS. Since C'2 activity decays rapidly at higher temperatures (11), it is imperative to keep these cells at 1° C before use.

The activity of this complex can be measured by lysis in guinea pig serum diluted in Na_3HEDTA–BBS, which chelates Ca^{++} and Mg^{++}, thereby blocking the action of C'1, C'4, and C'2, but permitting the reaction of C'3. In converse fashion these cells can be used to quantify the C'3 activity of a given reagent.

$EAC'_{1,4}$. This persensitized cell was prepared by inactivating the C'2 activity of $EAC'_{1,4,2}$ in BBS at 37° C for 90 minutes (12). The activity of this complex can be measured by lysis in guinea pig serum diluted in Na_2Mg EDTA–BBS. Na_2Mg EDTA chelates Ca^{++} but not Mg^{++}, thereby blocking the action of C'1, but permitting C'2 and C'3 to function. These cells can be used to quantify the combined C'2 and C'3 activity of a given reagent. In certain experiments $EAC'_{1,4}$ were prepared from C'x (8). C'y (diluted in BBS) was also used to study $EAC'_{1,4}$ activity.

EAC'_4. This persensitized red cell having the activity of C'4 was prepared by eluting C'1 from $EAC'_{1,4}$ (13). The elution was performed twice by incubating $EAC'_{1,4}$ in Na_3HEDTA–BBS for 15 minutes at 37° C. The EAC'_4 were then washed once in Na_3HEDTA–BBS, and twice in BBS, and were finally resuspended in the latter reagent. This complex, which will not lyse in Na_2Mg EDTA guinea pig serum, will lyse in R4.

EAC'_1. This intermediate complex was formed by the reaction of EA (sensitized in Na_3HEDTA–BBS, washed and resuspended in BBS) with R4 (14). Ten ml EA (5×10^8 per ml) was allowed to react with 1 ml R4 at 1° C for 10 minutes. The cells were then washed 3 times in the cold and resuspended in BBS. The activity of these cells was quantified by measuring the extents of their hemolysis in R1. EAC'_1 will also lyse in guinea pig serum diluted in Na_2Mg EDTA–BBS.

The effect of poly I on the formation and lysis of persensitized cells. Three general problems were investigated: *a*) Will poly I prevent the formation of a given persensitized cell? *b*) Will a given persensitized cell lose its activity if it is subsequently exposed to poly I? *c*) Will poly I, if added to the C' reagent used to test the lytic capacity of a persensitized cell, act to inhibit such lysis?

In all instances care was taken to use an excess of poly I calculated in terms of the number of $C'H_{50}$ present in an equivalent amount of whole guinea pig serum (1). The details of each experiment will be presented subsequently. The lytic experiments involving persensitized red cells were all carried out at 32° C for 60 minutes.

C'1 esterase. Human C'1 esterase prepared from a crude C'1 fraction (15) was further purified by DEAE and TEAE cellulose chromatography (16); the latter preparation contained 233 esterase units per ml. Highly purified C'1 esterase serum inhibitor was prepared by the method of Pensky (17). Estimation of C'1 esterase activity was done by microformol titration using ALTEE as a substrate (18, 19).

Density gradient ultracentrifugation. Since aggregated gamma globulin is known to possess anti-C' properties (20), mixtures of purified 7 S gamma globulin [4] and poly I were subjected to ultracentrifugation in a sucrose density gradient to determine whether poly I, *a*) formed a complex with gamma globulin, or *b*) caused its aggregation. The procedure has been described earlier (21).

The effects of poly I on C' activity in vivo. Groups of six male Wistar rats weighing between 150 and 300 g were injected with varying amounts of poly I dissolved in Tris-buffered saline, pH 7.4; the animals were then bled

[4] Cohn Fraction II, Cutter Laboratories, Berkeley, Calif.

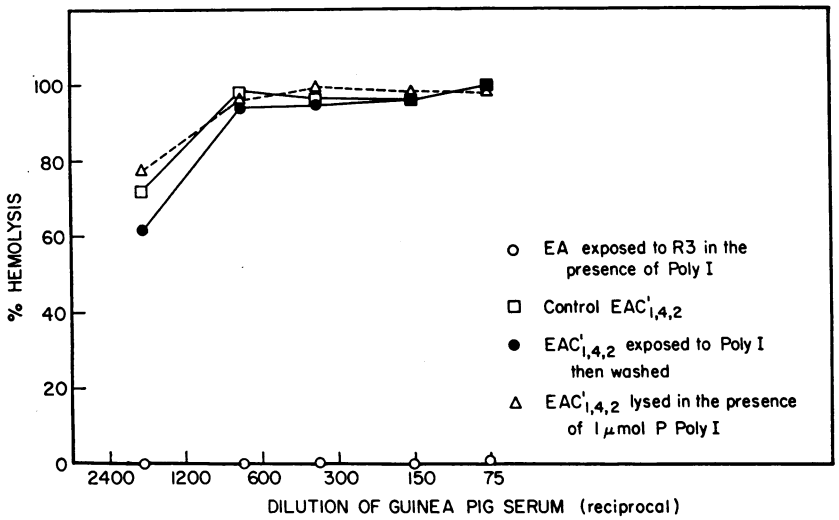

FIG. 1. EFFECT OF POLYINOSINIC ACID ON THE FORMATION AND LYSIS OF $EAC'_{1,4,2}$. 5×10^8 persensitized sheep red cells were subjected to lysis in 7.5 ml of varying dilutions of guinea pig serum diluted in barbital-buffered saline (BBS) containing 7.5×10^{-3} M Na_3HEDTA. (See text, footnote 1, for abbreviations.)

(0.5 to 0.8 ml) in serial fashion by cardiac puncture; the sera were separated within an hour and were pooled for each time period. A control group of six animals was injected with an equal volume of Tris-buffered saline, and they were bled in an identical fashion. All pooled serum samples, including a preinjection pooled sample for both experimental and control groups, were then assayed for C′ activity by the technique of Kabat and Mayer (*7b*). All assays were carried out in BBS free of Ca^{++} and Mg^{++}, since the presence of divalent cations lowers the C′ titer of Wistar rat serum by a significant amount (22). C′ assays were carried out within 2 hours of bleeding, and the sera were kept on ice during the intervening time. Wistar rat serum C′ activity was found to be labile to prolonged standing and to freezing and thawing. Preliminary *in vitro* studies showed that poly I was a potent inhibitor of rat serum C′ activity.

Results

I. *The effect of poly I on formation and lysis of persensitized cells*

$EAC'_{1,4,2}$. $EAC'_{1,4,2}$ were prepared as described. To study the effect of poly I on $EAC'_{1,4,2}$ formation, 2 μmoles P poly I was added per 10 ml EA and 0.5 ml R3. A 10-ml sample (in BBS) of washed $EAC'_{1,4,2}$ formed in the absence of poly I was subsequently exposed to 2 μmoles P poly I for 30 minutes at 1° C, washed 3 times, and resuspended in Na_3HEDTA–BBS. One-ml samples (5×10^8 cells per ml) of these two cell suspensions were added to 6.5-ml serial dilutions of guinea pig C′ in Na_3HEDTA–BBS and incubated at 32° C for 60 minutes. Control $EAC'_{1,4,2}$ cells were also added to similar C′ dilutions. A fourth test series utilizing control $EAC'_{1,4,2}$ cells was set up in identical C′ dilutions containing 1 μmole P poly I per test.

This experiment was performed on two separate occasions and the results were identical. One of these experiments is graphically presented in Figure 1. Exposure of $EAC'_{1,4,2}$ to poly I does not affect their susceptibility to hemolysis, nor does the presence of poly I in the diluted guinea pig C′ affect C′3 activity. The poly I that is present during the exposure of EA to R3 completely inhibits the formation of $EAC'_{1,4,2}$. The failure to form functional $EAC'_{1,4,2}$ might be attributed to acceleration of the decay reaction of $EAC'_{1,4,2}$ to $EAC'_{1,4}$. Accordingly, the effect of poly I on the rate of decay of $EAC'_{1,4,2}$ at 37° C was studied. The results (Figure 2) indicate that poly I has no effect on the temperature dependent rate of decay of $EAC'_{1,4,2}$ to $EAC'_{1,4}$.

$EAC'_{1,4}$. $EAC'_{1,4}$ were prepared as described earlier. To study the effect of poly I on $EAC'_{1,4}$ formation, 2 μmoles P poly I was added per 10 ml EA during the exposure to R3 at 1° C. The control $EAC'_{1,4}$ cells were split into two portions, one of which was incubated for another 30 minutes at 37° C in BBS, while the other was incubated un-

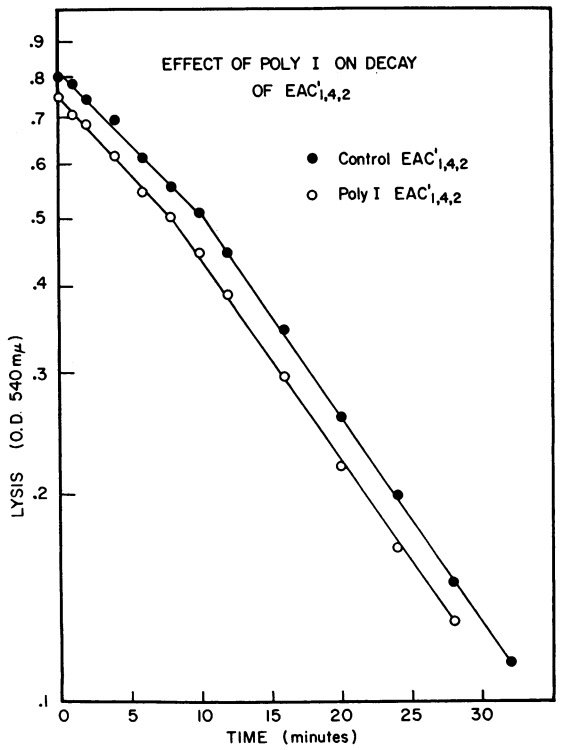

FIG. 2. EFFECT OF POLYINOSINIC ACID ON THE DECAY OF $EAC'_{1,4,2} \rightarrow EAC'_{1,4}$ AT 37° C. Two samples (18 ml) $EAC'_{1,4,2}$, one containing 4 μmoles P poly I, were placed in a 37° C waterbath. When they reached 37° C (0 time), 1-ml samples were removed at the designated intervals and added to 4 ml of a 1:160 dilution of guinea pig serum in Na_3HEDTA–BBS. Lysis was performed at 32° C for 60 minutes.

der the same conditions in the presence of 2 μmoles P poly I per 10 ml 5×10^8 $EAC_{1,4}$ per ml. The latter two cell suspensions were then washed 3 times in BBS and resuspended in BBS. All three cell suspensions were then subjected to hemolysis in serial dilutions of guinea pig serum in Na_2Mg EDTA–BBS as described for $EAC'_{1,4,2}$. $EAC'_{1,4}$ not previously exposed to poly I were tested for lysis in identical dilutions of guinea pig C′ to which 1 μmole P poly I per tube had been added.

Figure 3 illustrates the results obtained in one of three such experiments; the results in the other two were comparable. Poly I completely inhibits the formation of $EAC'_{1,4}$. Exposure of $EAC'_{1,4}$ to poly I, followed by washing, greatly inhibits subsequent lysis of $EAC'_{1,4}$ in Na_2Mg EDTA guinea pig serum; lysis of $EAC'_{1,4}$ is inhibited almost to the same extent by the presence of poly I during the actual lytic step. Similar experiments performed twice with $EAC'_{1,4}$ formed with C′x and tested for lytic capacity in C′y yielded identical results.

EAC'_4. During the preceding experiments on $EAC'_{1,4}$ we found that poly I prevented the formation of cells having EAC'_4 activity, since EA treated with R3 in the presence of poly I failed to lyse in R4. Accordingly, the experiments were limited to the effect of poly I on EAC'_4 formed in the absence of poly I.

EAC'_4 were exposed to 2 μmoles P poly I (per 10-ml cell suspension) for 15 minutes at 37° C and were then washed 3 times. These cells were subjected to lysis in a total volume of 7.5 ml of varying dilutions of R4 in BBS. Control EAC'_4 cells were similarly tested; in addition, EAC'_4 cells were subjected to lysis in identical R4 dilutions containing 1 μmole P poly I.

Exposure of EAC'_4 cells to poly I followed by washing did not affect their subsequent lysis in R4. The presence of poly I during the lytic phase, however, did inhibit EAC'_4 lysis (Figure 4).

EAC'_1. Ten ml (5×10^8 per ml) EA sensi-

TABLE II

The effect of polyinosinic acid (poly I) on C′1 esterase activity*

	Sample	Poly I present	C′1 esterase activity	Average	Inhibition
		μmoles	*U*		%
A†	1		23.2	22.4	
	1′		21.5		
	2	1	24.7	24.0	0
	2′	1	23.2		
	3	5	23.5	23.3	0
	3′	5	23.0		
B‡	1		23.7	23.1	
	1′		22.5		
	2	1	25.5	25.5	0
	2′	1	25.5		
	3	5	22.0	22.3	3.5
	3′	5	22.6		

* The C′1 esterase used in this experiment was a highly purified preparation containing 233 U per ml (undiluted).

† Poly I and substrate added together to enzyme at 0 time.

‡ Poly I and enzyme preincubated together at 37° C for 30 minutes before addition of substrate.

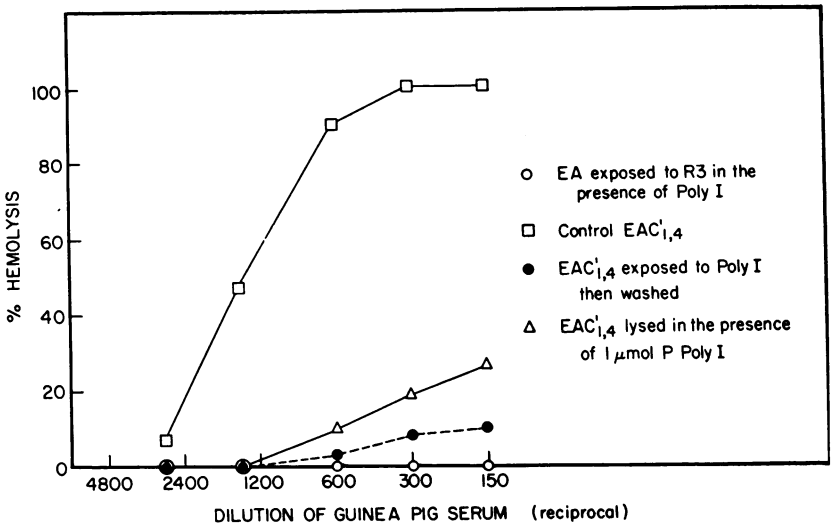

FIG. 3. EFFECT OF POLYINOSINIC ACID ON THE FORMATION AND LYSIS OF $EAC'_{1,4}$. The conditions and technique of the experiment were similar to those described in Figure 1. Lysis was carried out in varying dilutions of guinea pig serum in BBS containing 15×10^{-8} M Na_2Mg EDTA.

tized in Na_3EDTA–BBS was exposed to 1 ml R4 at 1° C for 10 minutes, both with and without 4 μmoles P poly I. After washing 3 times, a 10-ml sample of EAC'_1 formed in the absence of poly I was exposed to 4 μmoles P poly I for 30 minutes at 1° C, and the cells were again washed. The lytic tests were similar to those previously described, but the test reagent consisted of varying dilutions of R1 in BBS.

Poly I prevents the formation of EAC'_1. Control EAC'_1 cells exposed to poly I and subsequently washed, or subjected to lysis in R1 in the presence of 1 μmole P poly I, were almost completely inhibited (Figure 5).

II. *Studies on C'1 esterase and C'1 esterase serum inhibitor*

The experiments outlined in Tables II and III show that poly I is not capable of inhibiting C'1

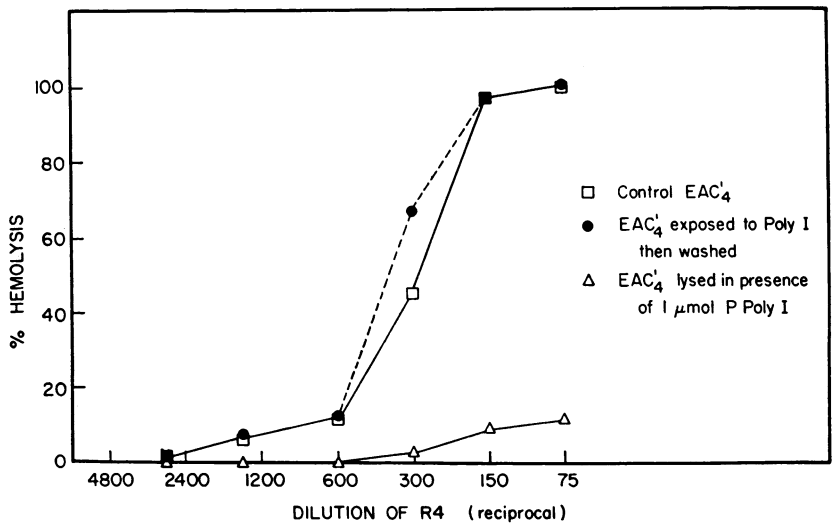

FIG. 4. EFFECT OF POLYINOSINIC ACID ON THE LYSIS OF EAC'_4 IN VARYING DILUTIONS OF R4. (5×10^8 EAC'_4; total volume per test, 7.5 ml).

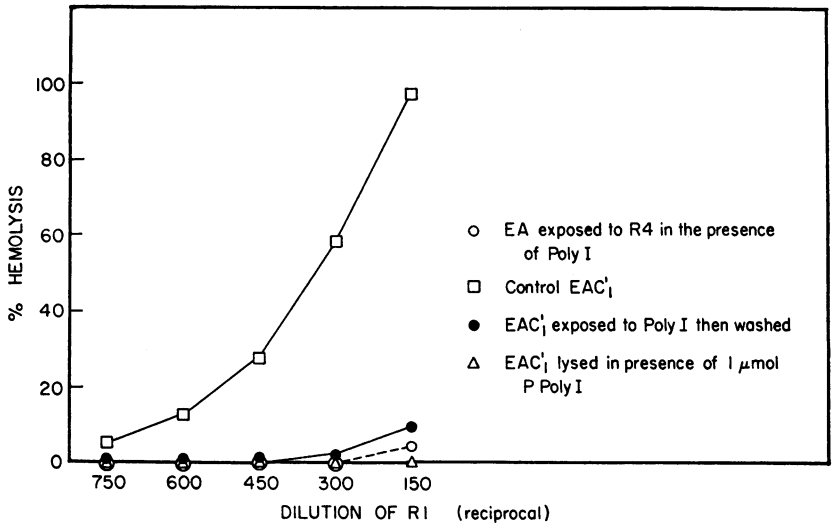

FIG. 5. EFFECT OF POLYINOSINIC ACID ON THE FORMATION AND LYSIS OF EAC'1. 5×10^8 cells and 7.5 ml of varying dilutions of R1 were used for each test.

esterase activity, nor can it prevent the inhibition of C'1 esterase by C'1 esterase serum inhibitor.

III. *Density gradient ultracentrifugation of polyinosinic acid and human 7 S gamma globulin*

When the sedimentation pattern of a mixture of poly I and 7 S gamma globulin in a sucrose density gradient is compared with the pattern for each substance analyzed separately under the same condition, no gross evidence for interaction between the two, or of aggregation of one by the other, can be seen (Figure 6).

TABLE III

The effect of poly I on the inhibition of C'1 esterase activity by C'1 esterase serum inhibitor†*

Sample no.	C'1 esterase serum inhibitor	Poly I present	C'1 esterase activity	Inhibition of esterase activity
		μmole	*U*	%
1	+	0	0.9	96
2‡	+	1	0	100
3		0	20.4	
4		1	20.3	0.5

* C'1 esterase was a crude preparation containing 40 U per ml.

† C'1 esterase serum inhibitor was a highly purified preparation containing 110 U per ml. 0.14 ml of a 1:10 dilution of this material was used wherever noted.

‡ Poly I and C'1 esterase serum inhibitor were preincubated for 30 minutes at 37° C before addition of C'1 esterase and substrate.

IV. *Effect of poly I on C' activity in vivo*

When administered by iv injection to Wistar rats in doses of 10 μmoles P, poly I consistently caused a precipitous decline in C' activity; by the end of 2 hours C' activity had returned to > 50% of the preinjection level (Table IV, Figure 7). Five μmoles poly I given intravenously to several experimental groups of animals yielded varying results ranging from no effect (one group) to 50% inhibition of C' activity (2 groups) and on one occasion to 90% inhibition of C' activity at the 15-minute bleeding. Intraperitoneal doses as high as 15 μmoles P poly I failed to cause a consistent significant fall in C' titer, although partial (50%) inhibition was occasionally seen. The poly I injections did not seem to affect the animals adversely; detailed pharmacologic studies, however, were not carried out.

Discussion

The results of these studies, examined within the framework of our present understanding of the C' system, suggest that poly I has a specific inhibitory action on C'1. The experiments with $EAC'_{1,4,2}$ clearly indicate that poly I has no inhibitory effect on C'3 activity. The fact that $EAC'_{1,4,2}$ exposed to poly I, and subsequently washed, retained full hemolytic susceptibility to C'3 indicates that poly I cannot inactivate cell-

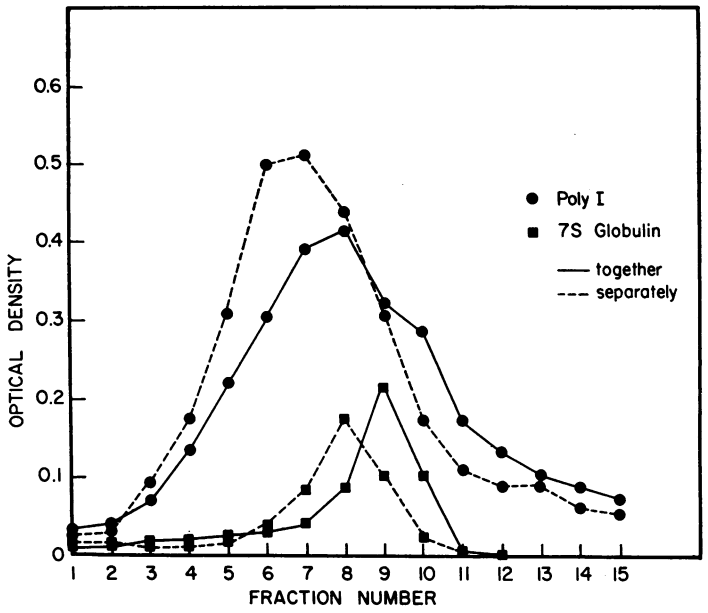

FIG. 6. THREE 10 TO 40% SUCROSE DENSITY GRADIENTS PREPARED WITH TRIS-BUFFERED SALINE (pH 7.4) AS SOLVENT. Solutions of 7 S gamma globulin (3 mg per ml), poly I (2 μmoles P per ml), and a mixture of 7 S globulin and poly I (same concentrations) were prepared. 0.2 ml of each solution was layered over one of the gradients, and all three were spun simultaneously for 16 hours, at 4° C, 38,000 rpm, in the SW-39 head of a Spinco Model L ultracentrifuge. After termination of the run, fractions were collected dropwise from the bottom of the tube (1 = bottom, 15 = top) and were analyzed for polynucleotide (OD, 249 mμ) and protein (Folin; OD, 660). ● = poly I, ■ = 7 S globulin; --- spun separately, —— spun as mixture.

bound C'2 or C'4. Even if it were inactivating cell-bound C'1 activity, this should not affect the susceptibility of these cells to lysis in C'3, since Becker has shown that $EAC'_{4,2}$ retain full lytic capacity in a C'3 reagent, despite the absence of C'1 (23). The inhibition of lysis of $EAC'_{1,4}$ by poly I present during the actual lytic phase might imply an inhibitory effect on C'2, but the fact that the same inhibition is obtained by pre-exposure of $EAC'_{1,4}$ to poly I, suggests that in fact a cell-bound activity is affected. Since EAC'_4 exposed to poly I are not inhibited from subsequent lysis in R4, and since active cell-bound C'1 is necessary for the attachment of C'2 to $EAC'_{1,4}$ (5, 23), the experiments with $EAC'_{1,4}$ point to C'1 as the most likely site of poly I inhibition. The experiments with EAC'_4 are consistent with this hypothesis, since, while cell-bound C'4 activity is not affected by poly I, the presence of the latter in R4 effectively inhibits EAC'_4 lysis. In contrast to EAC'_4, and in a manner analogous to $EAC'_{1,4}$, EAC'_1 are effectively inhibited by poly I, both by pre-exposure to poly I, and by its presence during the lytic test in R1. Since the attachment of C'1 to EA constitutes the first step in the activity of the C'

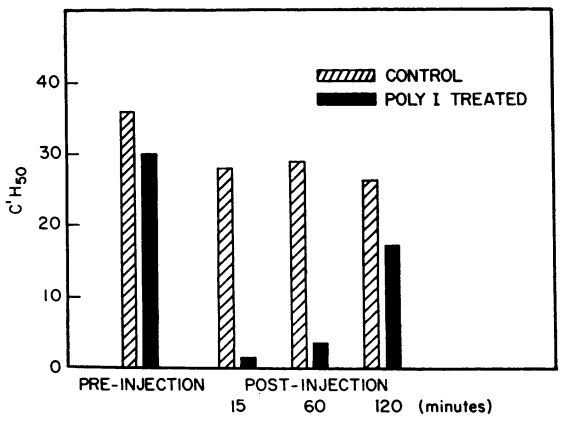

FIG. 7. EFFECT OF INTRAVENOUSLY ADMINISTERED POLYINOSINIC ACID ON THE COMPLEMENT LEVELS OF WISTAR RATS. A graphic representation of the data in Table IV.

TABLE IV

The in vivo effect of poly I on the complement titer of Wistar rats

Animal group*	Titer	Preinjection				Time—postinjection									
						15 minutes				1 hour				2 hours			
		OD 540	OD 540–blank†	Lysis‡	C'H$_{50}$	OD 540	OD 540–blank	Lysis	C'H$_{50}$	OD 540	OD 540–blank	Lysis	C'H$_{50}$	OD 540	OD 540–blank	Lysis	C'H$_{50}$
				%				%				%				%	
Control, 1 ml Tris saline iv	1:2	0.710	0.685	100		0.710	0.685	100		0.702	0.677	100		0.690	0.665	99	
	1:5	0.652	0.627	93		0.655	0.630	93		0.635	0.610	90		0.625	0.600	89	
	1:10	0.640	0.615	91	36	0.640	0.615	91	28	0.600	0.575	85	28.7	0.600	0.575	85	26.2
	1:20	0.580	0.555	82		0.525	0.500	74		0.532	0.507	75		0.500	0.475	70	
	1:40	0.315	0.290	43		0.187	0.162	24		0.200	0.175	26		0.164	0.139	21	
Experimental, 10 μmoles P poly I iv	1:2	0.690	0.665	99		0.132	0.107	16		0.495	0.470	70		0.690	0.665	99	
	1:5	0.655	0.630	93		0.066	0.041	6		0.263	0.238	35		0.640	0.615	91	
	1:10	0.640	0.615	91	30	0.038	0.013	2	1.4	0.070	0.045	7	3.4	0.550	0.525	78	17.2
	1:20	0.565	0.540	80		0.028	0.003	0		0.041	0.016	2		0.305	0.280	41	
	1:40	0.205	0.180	27		0.020		0		0.023		0		0.011	0.049	7	

* Six animals in each group, average wt, 191 g.
† Blank = 0.025.
‡ 100% lysis = OD 0.675.

system, it is not surprising that poly I, if indeed it is an inhibitor of C'1, can prevent the formation of EAC'$_1$, as well as the formation of all the subsequent intermediate complexes of guinea pig C' and EA. The inhibitory effect of poly I on the various persensitized red cells is analogous in every respect to the effect of C'1 esterase serum inhibitor on similar sensitized sheep red cell-C' intermediate complexes (5).

Although poly I can thus be considered as a potent inhibitor of C'1,[5] in a manner analogous to C'1 esterase serum inhibitor or DFP, it differs notably from these two C'1 inhibitors in having no inhibitory effect on C'1 esterase activity. The recent success of Lepow and his associates in fractionating C'1 activity into three subcomponents, of which C'1 (pro) esterase is only one (9), offers the option, currently being investigated, that poly I may interact specifically with one of the other two subcomponents in its inhibition of C'1.

Aggregated 7 S gamma globulin is a potent inhibitor of C' activity and has been shown by Marcus (25) to be an inhibitor of C'1. It was considered possible, therefore, that poly I might be anti-C' through the mediation of an aggregation of, or interaction with, 7 S gamma globulin. Although the sucrose density gradient ultracentrifugation experiments do not suggest any gross interaction such as that previously noted between polyribonucleotides and bovine serum albumin (21), the presence of a small amount of aggregate material cannot be definitely excluded by this technique. On the other hand, the inactivation of cell-bound C'1 activity on thoroughly washed persensitized red cells, where free 7 S gamma globulin is presumably lacking, makes the possibility that the anti-C' activity of poly I depends on the mediation of 7 S gamma globulin seem quite remote.

The experiments utilizing Wistar rats demonstrate that poly I, potent as an anti-C' agent *in vitro,* can also serve as an efficient decomplementing agent *in vivo* without any overt toxic effects to the animals. The amount needed to affect C' titers *in vivo* is somewhat larger than the theo-

[5] Exposure of EAC'$_1$ to as little as 8×10^{-4} μmoles P poly I per ml will result in 90% inhibition of lytic susceptibility (24).

retical amount calculated on the basis of *in vitro* data, and the duration of C′ activity inhibition *in vivo* is shorter than for other decomplementing agents such as aggregated gamma globulin (26). Nevertheless, its efficacy as an anti-C′ agent *in vivo* sustains the speculation that poly I may serve to protect against cellular injury in situations where C′ participation in immune reactions is involved.

Summary

The mechanism of the anticomplementary activity of polyinosinic acid has been investigated by its effect on the behavior of sensitized sheep erythrocyte-complement intermediates. Polyinosinic acid has been found to inhibit selectively the first component of complement (C′1). In contrast to other inhibitors of C′1, polyinosinic acid has no effect on C′1 esterase activity. The possibility that the anticomplementary properties of polyinosinic acid are mediated through some interaction with 7 S gamma globulin has been excluded with reasonable certainty. When injected intravenously into Wistar rats, polyinosinic acid is capable of precipitously lowering complement activity *in vivo*.

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