A STUDY OF THE CROSS-REACTIVITY OF ANTIPURIN-6-OYL SERUM WITH DEOXYRIBONUCLEIC ACID (DNA)*

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Rabbits immunized with purin-6-oyl–protein conjugates form antibodies with purine specificity. These antibodies are capable of fixing complement (C′) in the presence of thermally denatured deoxyribonucleic acid (DNA). This reaction was found to be specifically inhibited by purines and purine derivatives (1). Since the purines in DNA are linked to the deoxyribose-phosphate "backbone" at the 9-position, while the antipurinoyl antibody was elicited in response to an antigen in which the purine was coupled *via* the 6-position, it was not clear whether the specific reaction with DNA was characteristic of the total antipurinoyl antibody in the antisera, or whether this reaction was typical of a relatively small proportion of the antibody. This report shows that the reactions of antipurinoyl sera with DNA and with purinoyl-protein antigens are similar in specificity. In addition, it contains a demonstration of the reaction between antipurinoyl sera and thermally denatured DNA by the techniques of radioimmunoelectrophoresis (2, 3) and passive cutaneous anaphylaxis (4).

Materials and Methods

Proteins.—Bovine serum albumin (BSA), human serum albumin (HSA), and rabbit serum albumin (RSA) each were obtained as fraction V from Pentex, Inc., Kankakee, Illinois. Purin-6-oyl–BSA (Pur-BSA) and purin-6-oyl–HSA (Pur-HSA) were synthesized by the addition of 6-trichloromethylpurine (5, 6) to BSA and HSA, respectively, at pH 10.5. The details of these syntheses and the characterization of the conjugates have been described previously (1); the Pur-BSA and Pur-HSA conjugates contained, respectively, an estimated 24 and 27 purinoyl residues per molecule of serum albumin (1).

Nucleic Acids.—Pneumococcal DNA was prepared by the method of Hotchkiss (7). Calf thymus DNA was obtained from Mann Research Labs, New York (Lot A 6379). T5 bacteriophage DNA-C^{14} (specific activity = 1.34 μc/mg), prepared from *Escherichia coli* grown in a medium containing radioactive purines and pyrimidines, was generously supplied by Dr.

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Aaron Bendich, Sloan-Kettering Institute, New York, for use in the radioimmunoelectrophoresis experiments.

Pneumococcal DNA, in concentrations of 1 to 10 μg/ml in isotonic NaCl buffered at pH 7.5 with veronal or tris, was denatured by heating for 10 minutes in a boiling water bath followed by immediate chilling. The T5 DNA-C^{14} was denatured at concentrations of 190 to 200 μg/ml. The denaturation of DNA used in passive cutaneous anaphylaxis experiments is described separately under Immunochemical Procedures.

Deoxyribonuclease Treatment.—Pneumococcal DNA (15.8 μg/ml) was incubated for 1 hour at 37°C with pancreatic DNAase (Worthington Biochemical Corp., Freehold, New Jersey; 6.0 μg/ml) in an isotonic tris-buffered saline solution, pH 7.5, containing 5.2×10^{-3} M Mg^{++} and 7×10^{-5} M Ca^{++}.

Haptens.—*N*-Purin-6-oyl-ϵ-aminocaproic acid and *N*-purin-6-oyl-β-alanine were synthesized by the method of Cohen, Thom, and Bendich (6). Purine-6-carboxylic acid methyl ester (5), *N*-purin-6-oyl-glycine (6), purine-6-carboxamide (8), and 9-methylpurine (9) were kindly supplied by Dr. Sasson Cohen and Dr. Aaron Bendich. All other haptens used were commercial products of standard purity.

Immunochemical Procedures.—Rabbits were injected once weekly for 3 weeks into the foot-pads (10) with Pur-BSA or Pur-HSA in complete Freund's adjuvant mixture. Beginning 5 to 7 days after the last injection, the rabbits were bled three times by cardiac puncture and were finally exsanguinated 14 days after the last injection. All twenty-six animals immunized produced antibodies which precipitated with both purinoyl-protein conjugates.

Globulin fractions were prepated by a modification (11) of the sodium sulfate precipitation method of Kekwick (12).

Antipurinoyl antibody was purified by the method developed by Mage, *et al.* (13) for obtaining antiglucosyl and antigalactosyl antibodies, as follows: Enough Pur-HSA was added to E22-41 anti-Pur–BSA to precipitate all of the antibody (60 μg Pur-HSA nitrogen per ml antiserum). After washing, the specific precipitate was dissolved by addition of excess purin-6-oyl-β-alanine (Pur-β-Ala) in 0.01 M phosphate solution, pH 8.5. The supernatant of the resulting mixture was chromatographed on a column (2 x 40 cm) of DEAE–sephadex A-25, previously equilibrated with 0.01 M phosphate buffer, pH 7.1. Pur-HSA and Pur-β-Ala were retarded in their passage and the first peak of ultraviolet (280 mμ)–absorbing material which passed through the column consisted mainly of antibody. This eluate was lyophilized, redissolved in a small volume of water, and chromatographed on a column (2 x 40 cm) of sephadex G-25 (medium) equilibrated with 0.01 M phosphate, pH 7.2. The first peak of 280 mμ–absorbing material was collected. This solution was dialyzed against running tap water for 6 hours and was then lyophilized. Spectroscopic analysis indicated that the final product was free of hapten. It contained only γ-globulin detectable on immunoelectrophoretic analysis. This preparation of purified antipurinoyl antibody was 78 per cent precipitable upon the addition of Pur-HSA at equivalence.

Quantitative precipitin data were obtained using the Folin-Ciocalteu color reaction for analysis of specific precipitates (14). Hapten inhibition studies of precipitin reactions were carried out in the equivalence zone. All haptens used in precipitin inhibition studies were dissolved in tris-buffered saline (0.02 M tris in 0.85 per cent saline) at pH 7.5.

C′ fixation studies were performed using the quantitative method of Wasserman and Levine (15); 7.0 ml reaction volumes were employed. A veronal buffer (16), containing 0.1 per cent BSA or RSA, was used in some early experiments. However, to avoid any possible artifacts that might have arisen from the presence of veronal, which is formally a hexahydropyrimidinetrione, a tris-HCl buffer was used in most of the experiments reported herein. The tris-HCl buffer was made as follows: 2 liters of a stock solution were prepared containing 81.6 gm NaCl, 12.1 gm tris [tris(hydroxymethyl)aminomethane; Sigma Chemical Company,

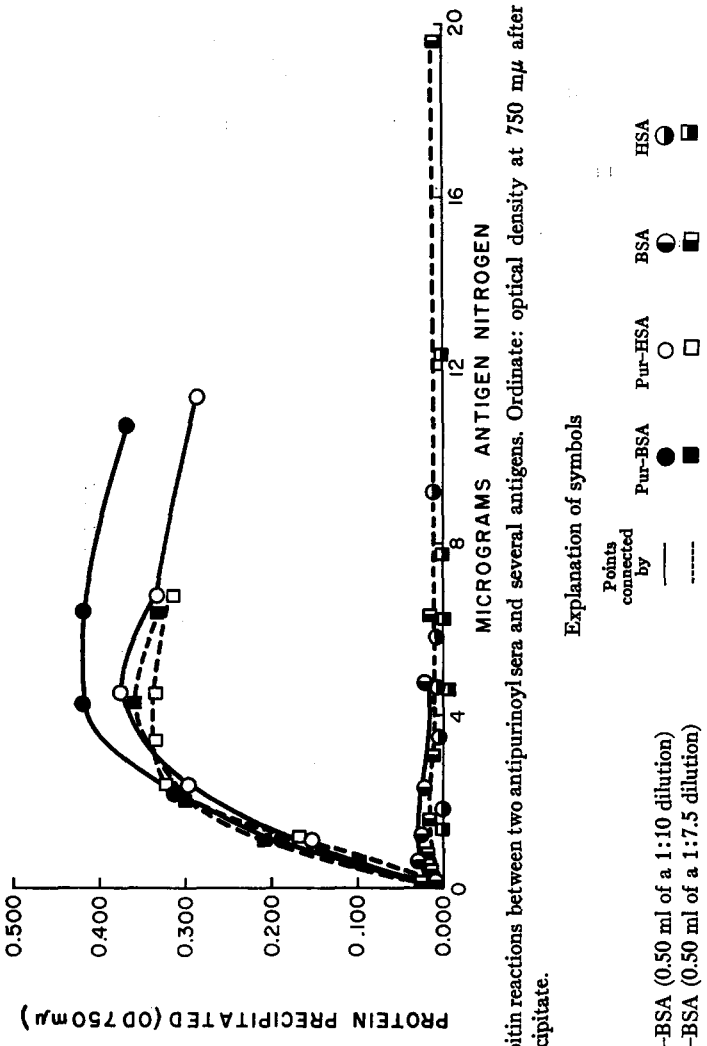

FIG. 1. Precipitin reactions between two antipurinoyl sera and several antigens. Ordinate: optical density at 750 mμ after Folin-Ciocalteu reaction on precipitate.

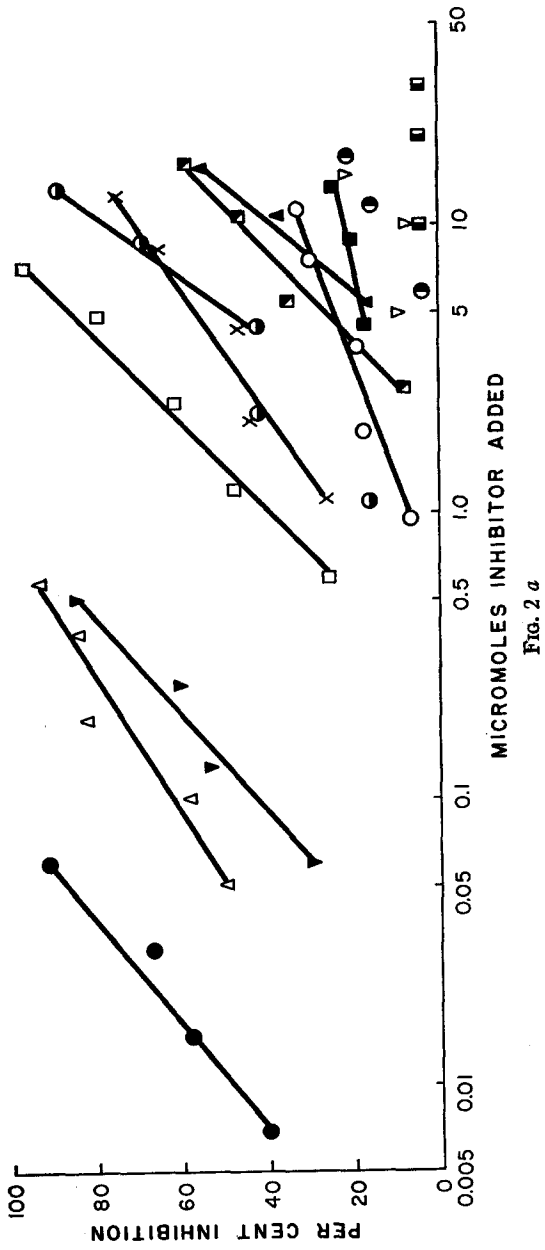

FIG. 2 *a*

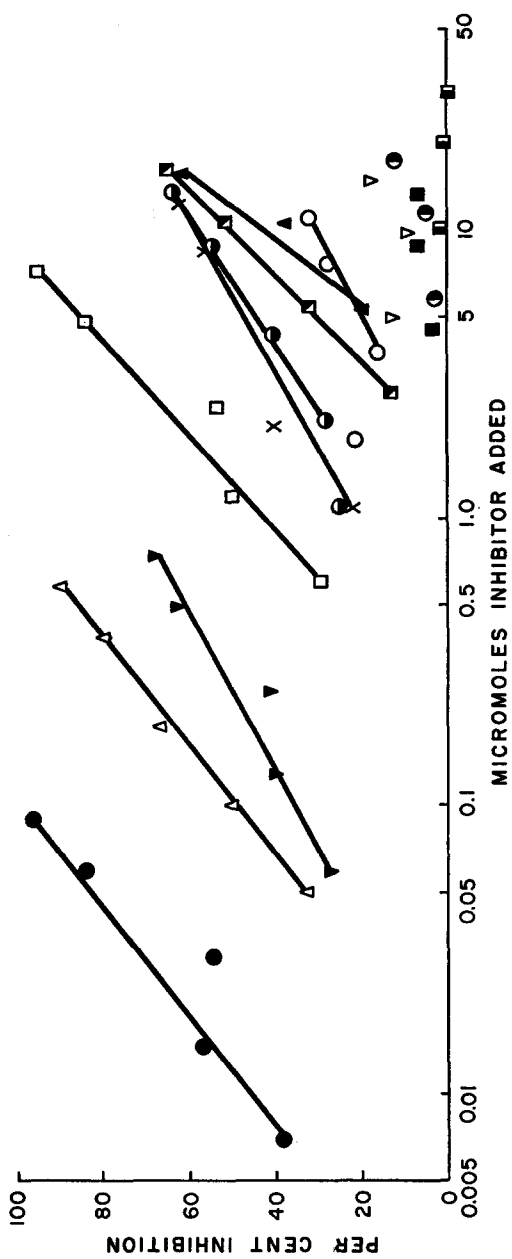

FIG. 2 *b*

FIGS. 2*a* and 2*b*. Hapten inhibition of precipitin reactions between Pur-HSA and antipurinoyl sera. Ordinate: Per cent inhibition of precipitin reaction between (*a*) 8.8 μg Pur-HSA nitrogen and 0.50 ml of a 1:10 dilution of a globulin fraction of antiserum E22-27, and (*b*) 6.6 μg Pur-HSA nitrogen and 0.50 ml of a 1:4 dilution of a globulin fraction of antiserum E22-30.

St. Louis] and enough 2 N HCl (40 to 42 ml) to adjust the pH to 7.45–7.55. The stock solution was diluted 1:5 and RSA was added to a concentration of 0.1 per cent, Mg^{++} to 5×10^{-4} M and Ca^{++} to 1.5×10^{-4} M for C′ fixation studies. DNA (prior to denaturation only) and haptens were dissolved in the buffer without the addition of RSA.

Immunoelectrophoresis experiments were performed on microscope slides according to the method of Scheidegger (17). Duck anti-rabbit serum used in these experiments was kindly supplied by Dr. Charles L. Christian, Columbia University, New York.

In the radioimmunoelectrophoresis experiments, DNA-C^{14} was added together with the duck antiserum to the antiserum troughs. After 72 hours, the slides were washed several times, dried, and affixed to strips of Kodak no-screen x-ray film for 5 to 14 days. The autoradiograms were then developed and compared with the immunoelectrophoretic precipitates.

Passive cutaneous anaphylaxis (PCA) was carried out as described by Ovary (4). Guinea pigs were injected intradermally with 0.1 ml aliquots of 1:100, 1:1000 and 1:10,000 dilutions of antipurinoyl serum. Five hours later they were challenged by the intravenous injection of 0.5 ml of antigen solution mixed with 0.5 ml of 1 per cent Evans blue dye. Thirty minutes after this challenge, the reactions were read and recorded as the diameter of visible intradermal extravasation of Evans blue dye. An extravasation 5 mm or more in diameter was interpreted as a positive reaction. Difficulties were encountered in the denaturation of DNA for this procedure because the concentrations required are greater than those at which optimal denaturation of DNA can be achieved. The calf thymus DNA was denatured at a concentration of 2.5 mg/ml in 0.85 per cent saline and became gelatinous. Partial dispersion was achieved with a syringe and it was possible to inject this material intravenously (in this instance, 1.0 ml of the DNA preparation was injected with 0.5 ml dye). Pneumococcal DNA was denatured at a concentration of 2 mg/ml in aqueous solution and did not become gelatinous; after mixing with equal volumes of 1 per cent Evans blue dye (in 0.85 per cent saline), native and denatured pneumococcal DNA's were injected intravenously at final concentrations of 1 mg/ml.

RESULTS

Sera E22-27, E22-29, E22-30, E22-41 (anti-Pur–BSA) and E22-42 (anti-Pur–HSA) were studied extensively. Quantitative precipitin values obtained upon addition of various antigens to sera E22-27 and E22-29 are presented in Fig. 1. In both instances, relatively small amounts of antibody were precipitated by BSA or HSA compared with the much larger precipitates obtained with Pur–BSA or Pur–HSA. This has been noted with all antipurinoyl sera studied to date. The small amount of antibody formed to the "backbone" protein of the antigens may be due to the relatively large numbers of purinoyl residues in the conjugates.

For further characterization of the specificity of these antisera, the ability of various haptens to inhibit the precipitin reactions between Pur–HSA and three anti-Pur–BSA globulin preparations was studied. In Figs. 2 *a* and 2 *b* the data are recorded as the per cent inhibition, by given amounts of hapten, of the precipitin reactions between Pur–HSA and the globulin fractions from antisera E22-27 (Fig. 2 *a*) and E22-30 (Fig. 2 *b*). In Table I are listed the quantities of each hapten required for 50 per cent inhibition of the precipitin reactions between Pur–HSA and globulins from antisera E22-27, E22-29, and E22-30. It

can be seen from Figs. 2 *a* and 2 *b* and Table I that the relative order of effectiveness of haptens as inhibitors of these precipitin reactions showed but minor variations among the three antisera studied. *N*-Purin-6-oyl-ϵ-aminocaproic acid was the most effective of all the haptens studied. All compounds containing the purin-6-oyl structure were extremely effective inhibitors. In general, most other purines and purine derivatives were moderately good inhibitors whereas pyrimidines were relatively poor inhibitors. Although it has been reported that

TABLE I

Quantity, in μmoles, of Various Haptens Required to Produce 50 Per Cent Inhibition of the Precipitin Reaction between Pur-HSA and Anti-Pur-BSA at Equivalence*

Hapten	Anti-Pur-BSA		
	E22-27	E22-29	E22-30
	μmoles	*μmoles*	*μmoles*
N-Purin-6-oyl-ϵ-aminocaproic acid	0.01	0.005	0.01
N-Purin-6-oyl-glycine	0.05	0.36	0.10
Purine-6-carboxamide	0.13	0.4	0.25
Hypoxanthine	1.5	3.0	1.3
6-Methylpurine	3.6		6.0
Adenine	5.2	>8.0 (30)	7.0
Deoxyadenosine	12.0	>20.0 (29)	9.8
Uracil	14.0	>15.0 (3)	12.0
Adenosine	>11.4 (33)	>20.0 (27)	>11.4 (32)
9-Methylpurine	>13.4 (24)		>13.4 (7)
Thymine	>15.0 (21)	>20.0 (8)	>15.0 (17)
Cytosine	>17.3 (21)	>20.0 (10)	>17.3 (12)
ϵ-Aminocaproic acid	>30.2 (4)	>30.0 (0)	>30.2 (0)

* Instances where 50 per cent inhibition was not attained, largest quantity of hapten used is listed, followed, in parentheses by per cent inhibition achieved by that amount of hapten.

high concentrations of ϵ-aminocaproic acid are capable of inhibiting antigen-antibody reactions (18), no such effect was observed with the concentrations used in these experiments (Table I).

Antipurinoyl sera are capable of fixing C′ in the presence either of purinoyl-protein conjugates or of denatured pneumococcal DNA. These C′ fixation reactions have been compared in the case of each of the five sera studied. The ability of purinoyl-protein conjugates to fix C′ by reacting with each of these sera is shown in Fig. 3. The reactions of each of four antipurinoyl sera in C′ fixation with native and denatured pneumococcal DNA are shown in Fig. 4. In Table II are listed the highest dilutions of each antiserum which exhibited maximal C′ fixation in the 75 to 90 per cent range upon the addition of purinoyl-

protein conjugates or of denatured pneumococcal DNA. In all instances the titers were lower with DNA than they were with purinoyl conjugates.

It can be seen from the data presented in Fig. 4 that, as has been previously reported (1), the ability of native DNA to fix C′ was negligible, whereas a maximum C′ fixation of about 80 per cent was observed when denatured DNA was added to comparable dilutions of each antiserum. At dilutions of 1:100, antisera E22-29 and E22-30 showed some reactivity with native DNA (Fig. 5).

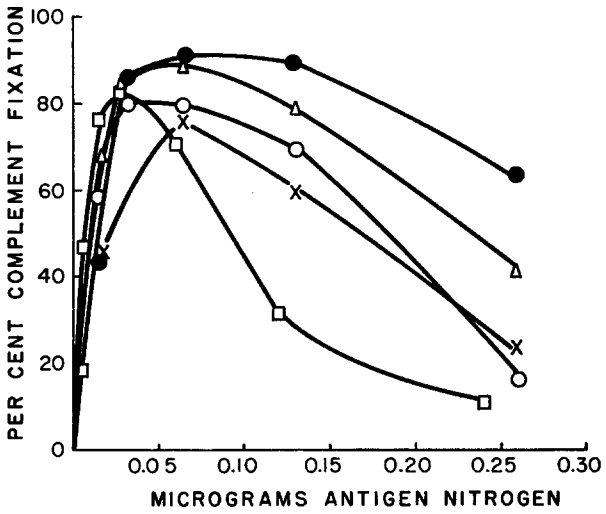

FIG. 3. C′ fixation reactions between purinoyl-protein conjugates and 1.00 ml of
E22-27 (1:600 dilution), ●
E22-29 (1:600 dilution), ○
E22-30 (1:800 dilution), ×
E22-41 (1:740 dilution), △
E22-42 (1:1200 dilution), □
Antigen was Pur-HSA except for E22-42, in which case Pur-BSA was used.

This reactivity was, however, slight when compared with the almost complete fixation of C′ observed with much smaller concentrations of denatured DNA, and may reflect the presence of traces of denatured DNA in the native preparation used. The anticomplementary properties of all antipurinoyl sera at dilutions of 1:75 and less prevented studies of this type with the other antisera.

In other experiments, pneumococcal DNA was treated with DNAase prior to denaturation. In all instances studied, the reaction of the denatured DNA was abolished by such prior treatment with DNAase.

The capacity of various haptens to inhibit the C′ fixation reaction between DNA and antiserum E22-29 is depicted in Fig. 6. The quantity, in micromoles, of each hapten required for 50 per cent inhibition of this reaction is listed in

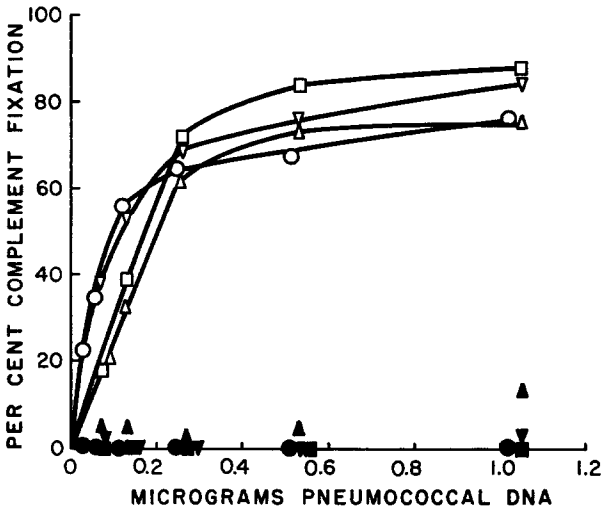

FIG. 4. C′ fixation reactions between native (closed symbols) and denatured (open symbols) DNA and 1.00 ml of

E22-27 (1:75 dilution), ■ □
E22-29 (1:350 dilution), ▲ △
E22-30 (1:400 dilution), ● ○
E22-41 (1:80 dilution), ▼ ▽

TABLE II

C′ Fixation Titers of Antipurinoyl Sera with Purinoyl-Proteins and with Denatured Pneumococcal DNA

Antiserum	Antipurinoyl antibody concentration	C′ fixation titers*		
		Pur–HSA	Pur–BSA	DNA
	μgN/ml			
E22-27 Anti-Pur–BSA	297	1:600		1:75
E22-29 Anti-Pur–BSA	199	1:600		1:350
E22-30 Anti-Pur–BSA	235	1:800		1:400
E22-41 Anti-Pur–BSA	183	1:740		1:80
E22-42 Anti-Pur–HSA	249		1:1200	1:80

* Titers are recorded as highest dilutions capable of producing maximal C′ fixation in the 75 to 90 per cent range.

Table III; similar data for serum E22-30, previously reported (1), are also provided for comparison. Comparable data for serum E22-27 could not be obtained with precision because of its marked anticomplementary properties. These data with serum E22-27 are therefore, best considered as approximate values (Table III). Again, as with the corresponding precipitation reactions with

Pur–HSA (Table I) purinoyl-ε-aminocaproic acid was invariably the best hapten inhibitor of the C′ fixation reactions with DNA (Table III). Although the relative quantities of other haptens required to inhibit the reaction with DNA varied from the quantities required to inhibit the reaction with Pur–HSA, the relative order of effectiveness of these haptens as inhibitors of the reaction with DNA (Table III) paralleled their order of effectiveness as inhibitors of precipitation with Pur–HSA (Table I) rather closely.

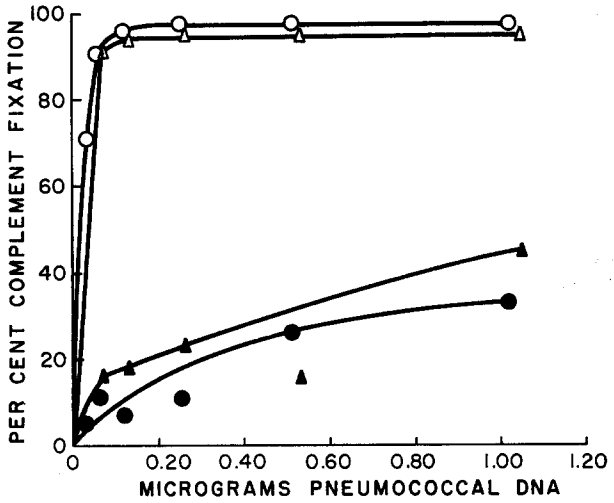

FIG. 5. C′ fixation reactions between native (closed symbols) and denatured (open symbols) DNA and 1.00 ml of 1:100 dilutions of

E22-29, ▲ △

E22-30, ● ○

Antipurin-6-oyl antibody was purified to demonstrate that the reactivity of antipurinoyl sera with DNA was an integral property of this antibody. The ability of the purified antibody preparation to fix C′ in the presence of denatured pneumococcal DNA is indicated in Table IV.

The reaction of antipurinoyl sera with DNA was demonstrated by yet another immunochemical method, namely that of radioimmunoelectrophoresis (2, 3). By this technique, reactivity of denatured, C^{14}-labeled, T5 bacteriophage DNA with the γ-globulin of antipurinoyl serum or with purified antipurinoyl antibody could be demonstrated (Fig. 7). In control experiments, the reaction of unheated preparations of this labeled DNA with the γ-globulin of antipurinoyl sera was not observed. Denatured radioactive DNA was not bound by the γ-globulin of sera obtained from normal animals or from animals immunized with other antigens. In several instances, both with control and with

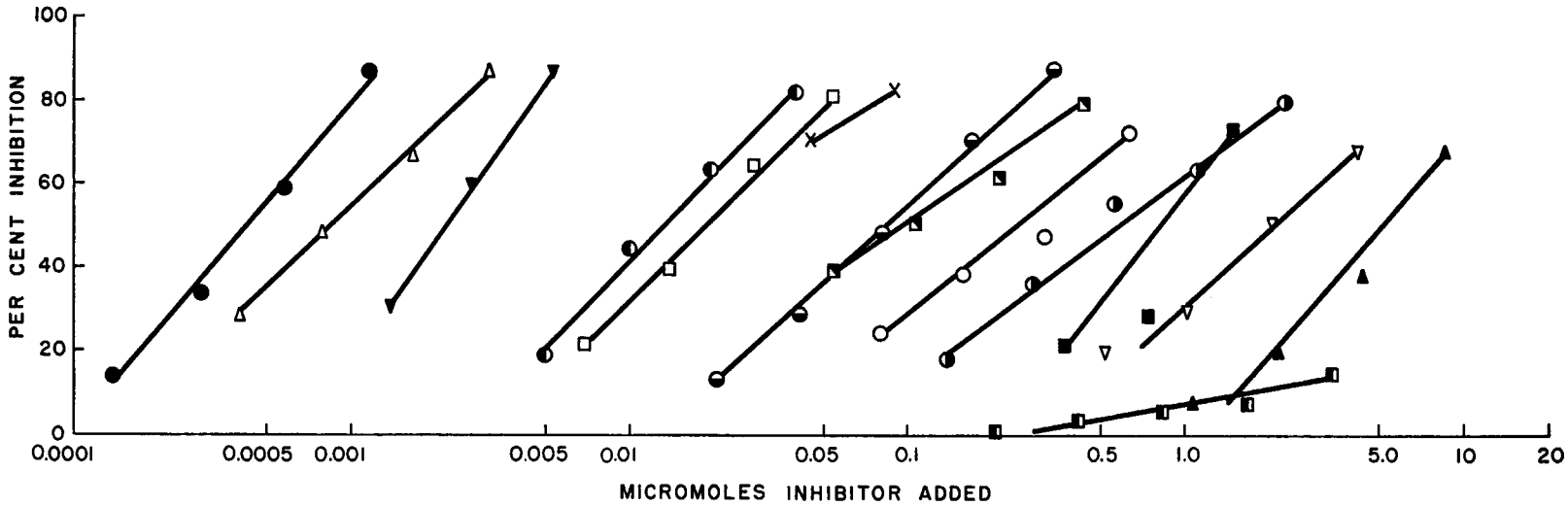

FIG. 6. Hapten inhibition of C′ fixation reaction between denatured pneumococcal DNA and antiserum E22-29. ◐ represents purine-6-carboxylic acid methyl ester. For explanation of all other symbols, see Fig. 2.

TABLE III

Quantity, in μmoles, of Various Haptens Required to Produce 50 Per Cent Inhibition of C' Fixation Reaction between Denatured Pneumococcal DNA and Anti-Pur–BSA Serum*

Hapten	Rabbit anti-Pur-BSA serum		
	E22-27‡	E22-29	E22-30§
	μmoles	*μmoles*	*μmoles*
N-Purin-6-oyl-ε-aminocaproic acid	<0.01 (85)	0.0004	<0.01 (100)
N-Purin-6-oyl-glycine	<0.014 (84)	0.0009	<0.02 (89)
Purine-6-carboxamide		0.002	0.01
Purine-6-carboxylic acid methyl ester	~0.04	0.01	<0.06 (85)
Hypoxanthine		0.02	<0.11 (75)
6-Methylpurine		<0.04 (70)	0.12
Adenine	~0.03	0.08	0.30
Deoxyadenosine	<0.22 (84)	0.10	0.70
Uracil	~3.0	5.2	>4.5 (40)
Adenosine		0.25	0.70
9-Methylpurine		0.84	1.0
Thymine		2.1	1.5
Cytosine	~1.5	0.60	5.4
ε-Aminocaproic acid		>3.4 (14)	

* In instances where 50 per cent inhibition was not observed, largest or smallest quantity of hapten tested is listed, followed, in parentheses, by per cent inhibition observed with that amount of hapten.

‡ Approximate values (See text for explanation).

§ From reference 1.

TABLE IV

Per Cent C' Fixation Observed on Addition of Denatured Pneumococcal DNA to Purified Anti-purin-6-oyl Antibody from Rabbit E22-41

Antibody N used	Denatured pneumococcal DNA added	
	0.51 μg	1.02 μg
μg	*per cent*	*per cent*
1.5	16	46
3.0	85	93

antipurinoyl sera, some binding of radioactivity was associated with an α_2-globulin which was not further identified.

The reaction of E22-41 anti-Pur–BSA with Pur–HSA and with pneumococcal or calf thymus DNA could further be demonstrated by the passive cutaneous

anaphylaxis (PCA) method (4). The results are shown in Table V. Significant reactivity with Pur-HSA was observed with a 1:10,000 dilution of this antiserum following the intravenous injection of 0.5 mg Pur-HSA. Reaction with both pneumococcal and calf thymus DNA's was noted with a 1:100 dilution of

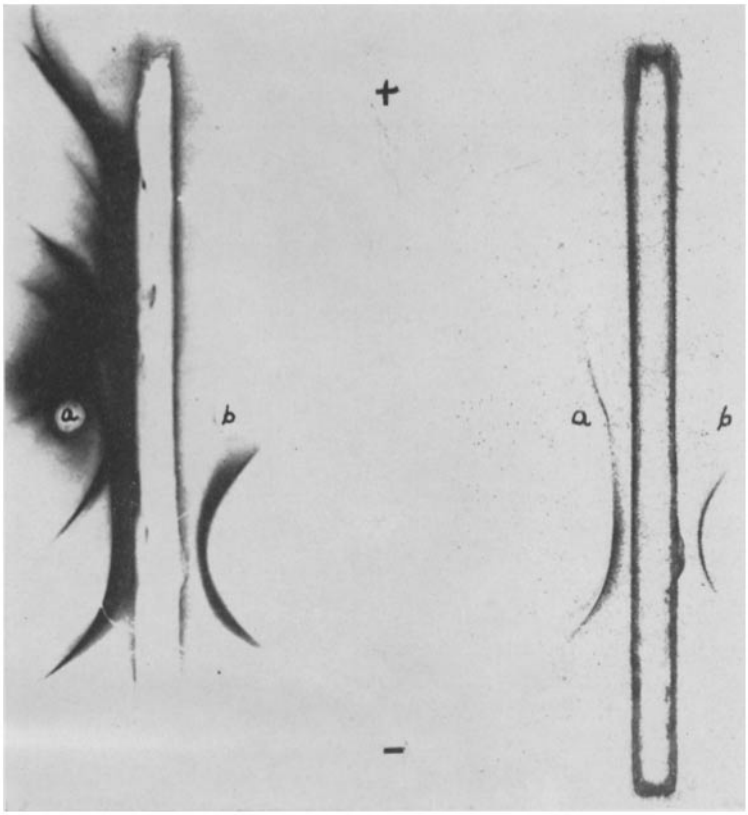

FIG. 7. Radioimmunoelectrophoresis. After electrophoresis of antiserum E22-30 (*a*) and purified antipurinoyl antibody (*b*) in agar, duck anti-rabbit serum and denatured T5 bacteriophage DNA-C^{14} were added to the center trough. Immunoelectrophoretic pattern is at left and corresponding autoradiogram is at right.

antiserum. It is of interest that native and denatured pneumococcal DNA's, in equal amounts (1.0 mg), elicited approximately equivalent PCA reactions with this antiserum. Whether this represents reaction of antibody with native DNA or merely reflects *in vivo* degradation of the injected native pneumococcal DNA preparation is not clear at this time.

TABLE V

*Passive Cutaneous Anaphylaxis Experiments with E22-41 Anti-Pur–BSA**

E22-41 serum dilution:	Antigen injected:																	
	Pur–HSA			HSA		Native pneumococcal DNA				Denatured pneumcoccal DNA				Denatured calf thymus DNA				
1:100	20	30	30	0	0	18	10	12	0	0	15	Trace	13	20	15			
1:1,000	15	25	18	0	0	0	0	0	0	0	0	0	0	0	0			
1:10,000	0	8	8	0	0	0	0	0	0	0	0	0	0	0	0			

* Results recorded as diameter (in millimeters) of area of extravasation of dye 30 minutes after intravenous injection of antigen-dye mixture. Each vertical column represents sites of three intradermal injections of dilutions of E22-41 antiserum in one guinea pig. Animals injected with DNA appeared to react somewhat more slowly than animals injected with Pur-HSA.

DISCUSSION

Rabbits immunized with purin-6-oyl–protein conjugates produce purine-specific antibodies (Figs. 1 to 3; Tables I, II); only relatively small quantities of antibody with specificity for the protein carrier (BSA or HSA) are formed (Fig. 1). The hapten inhibition data for the precipitin reactions between Pur–HSA and three antipurinoyl sera (Figs. 2 *a* and 2 *b*; Table I) clearly indicate that purin-6-oyl-ε-aminocaproic acid is the most effective inhibitor of this reaction yet tested. This observation strongly supports the assumption (1), based on spectrophotometric data and the preferential reactivity of 6-trichloromethylpurine with free amino groups at a mildly alkaline pH (1, 6), that the purin-6-oyl residues of the Pur-protein conjugates are linked by covalent peptide bonds *via* the free ε-amino groups of lysine. All compounds thus far studied which contain the purin-6-oyl structure are likewise potent inhibitors; they are more effective inhibitors than the purines themselves or other purine derivatives, which also implies that the conjugated linkage possesses the purin-6-oyl structure. As has been noted, other purine derivatives are moderately effective inhibitors of this reaction and pyrimidines possess a slight inhibitory capacity. This latter property is not unexpected, in view of the fact that the basic purine structure contains the pyrimidine ring.

It has been demonstrated that antipurinoyl sera fix C′ in the presence of thermally denatured pneumococcal DNA (Figs. 4, 5; Table II) and that this reaction of DNA with antipurinoyl sera can be abolished by prior treatment of the DNA with DNAase. Serum E22-30 has previously been shown (1) to fix C′ with other thermally denatured bacterial DNA's as well as with unheated, single-stranded (19) DNA from bacteriophage φX 174.

The reaction of antipurinoyl sera with denatured DNA is best explained (1)

in terms of current concepts of DNA structure (20), in which it is postulated that thermal denaturation cleaves weak hydrogen bonds between apposed purine and pyrimidine bases of the double helix, thereby separating the strands and rendering the purine bases of the DNA more accessible for reaction with antipurinoyl antibody. Such preferential reactivity with thermally denatured, single-stranded DNA was first noted by Levine and coworkers with rabbit antibodies to T-even bacteriophage DNA (21). In this instance the antibodies were shown to have specificity for an unusual glucosylated pyrimidine base, 5-hydroxymethylcytosine (22). A similar preferential reaction with single-stranded DNA has been described for the sera of many human patients with systemic lupus erythematosus (23, 24). Pyrimidine-specific rabbit antibodies which cross-react with DNA similarly exhibit greater reactivity with denatured DNA (25), although some reaction with native DNA is found. Recently, Timakov *et al.* (26) and Christian *et al.* (27) have detected, in the sera of rabbits hyperimmunized with bacteria, antibodies which react better with denatured DNA than with native DNA.

That the DNA-reactive antibody in the current study is purine-specific can be inferred from two observations,—first, the pattern of hapten inhibition (Table III; Fig. 6), and second, the ability of purified antipurin-6-oyl antibody to react with denatured DNA, as demonstrated by C′ fixation (Table IV) and radioimmunoelectrophoresis (Fig. 7). Prior to this work it was not certain whether the antibody in antipurinoyl sera which reacts with DNA is representative of the total antipurin-6-oyl antibody or whether it represents a small fraction of the antibody with different specificity. The hapten inhibition data (Table III; Fig. 6) indicate that purin-6-oyl amino acids are the best inhibitors of the C′ fixation reaction between these sera and DNA, and that the relative order of effectiveness of all haptens in this C′ fixation reaction closely parallels their relative order as inhibitors of the precipitin reaction between antipurinoyl sera and Pur–HSA (Table I). This observation indicates that the antibody which reacts with DNA is indeed representative of the total antipurin-6-oyl antibody in these sera. Of particular interest is the finding that 6-methylpurine is a better inhibitor than is 9-methylpurine of the reaction of antibody with DNA as well as of its reaction with Pur–HSA despite the fact that, as previously mentioned, purines in DNA are linked to deoxyribose at the 9-position. The relative effectiveness of these haptens thus depends on the specificity of the antibody, and not on the structure of the particular cross-reacting antigen used to measure the effect.

The titers of antipurinoyl sera were lower with DNA than they were with purinoyl-protein conjugates, both in C′ fixation (Table II) and PCA (Table V) experiments. This was expected since the reactions with DNA are cross-reactions. It should be noted that the ratio of the titers (with DNA and with the conjugate) indicates the *minimum* amount of cross-reacting antibody. The

amount of antibody involved in the reaction with DNA may actually be greater than that indicated by this ratio; for example, Osler and Heidelberger (28) have shown that about five times as much antibody may be required to fix a given amount of C′ when it reacts with a cross-reacting antigen than is required to fix the same amount of C′ when the antibody reacts with the homologous antigen.

It was found in the current study that 200 to 500 mμg precipitating antipurinoyl N were required to obtain maximal C′ fixation of 75 to 90 per cent with purinoyl-protein conjugates (*cf.* Table II). This finding is in accord with the observations of Reichlin, Wilson, and Levine (29), who found that 70 to 700 mμg antibody N are required to produce comparable C′ fixation with rabbit antisera, the differences being related to the immunization schedule and the antigen being injected. In the cases of certain antisera, 20 to 40 mμg antibody N have been sufficient to produce this degree of C′ fixation (15), but in these instances the antisera were obtained from hyperimmune animals (30). Similar variations in the C′-fixing ability of rabbit anti-BSA have been correlated by Wallace, Osler, and Mayer (31) with differences in immunization schedules.

A well delineated zone of antigen excess was noted in all C′ fixation reactions between purinoyl-protein conjugates and antipurinoyl sera (Fig. 3). In the curves depicting the C′ fixation reactions between these antisera and denatured pneumococcal DNA (Figs. 4 and 5), however, no zone of antigen excess is seen. Whether a decrease in the percentage of C′ fixation would be observed if larger quantities of DNA were used cannot be stated because quantities of DNA greater than 2 μg exhibit significant anticomplementary activity in the C′ fixation system used. Stollar and Levine (32) have encountered a few sera from patients with systemic lupus erythematosus which react maximally with relatively large amounts of denatured DNA (1 μg rather than 0.02 μg, which is optimal for most DNA-reactive lupus sera) and in which no zone of antigen excess can be demonstrated. These authors noted the similarity of the C′ fixation curves of these lupus sera with those of antipurinoyl sera and suggested that the requirement for a relatively large amount of DNA may reflect the specificity of antibody for a relatively small repeating determinant unit, such as a single base or a nucleotide (32).

Using the technique of radioimmunoelectrophoresis, C^{14}-labeled denatured DNA was shown to bind to the γ-globulin of antipurinoyl sera and to purified antipurinoyl antibody. Since the duck serum added with the C^{14}-DNA may have contained deoxyribonucleases, such as are present normally in the sera of many species (33, 34), the bound radioactivity could have represented either intact DNA or subunits derived from it. Sufficient radioactive DNA of satisfactory purity and specific activity was not available for further study of this question. An incidental finding in the course of these experiments was the

binding of radioactivity to the α_2-globulins of both normal and immune sera. The role, if any, of these proteins in the transport and metabolism of deoxyribonucleic acid or its subunits remains to be investigated.

The reactivity of antipurinoyl sera with DNA was further demonstrated by the PCA method (Table V). Native and denatured pneumococcal DNA's in equal concentrations elicited approximately equivalent PCA reactions. The basis for the difference in reactivity with native DNA by PCA as compared with that observed in C′ fixation and radioimmunoelectrophoresis is not apparent. It is possible, however, that different classes of antibodies are being detected by different methods. In this regard, the recent finding (35) that different classes of precipitating antibodies appear to be responsible in guinea pigs for C′ fixation and PCA, respectively, may be particularly relevant.

Recently it has been shown that antipurinoyl sera, as well as some other antipurine and antipyrimidine sera, precipitate with certain denatured DNA preparations (36). Neither precipitation nor C′ fixation between RNA and antipurinoyl sera has been detected, but RNA, thermally denatured in the presence of formaldehyde, can inhibit precipitation between the antipurinoyl sera and calf thymus DNA, also thermally denatured in the presence of formaldehyde (37). Some reactivity of antipurinoyl sera with RNA preparations was also detected by PCA and radioimmunoelectrophoresis. In these instances, however, contamination of the RNA with DNA has not yet been excluded as a possible basis for the reaction.

The reaction of antipurinoyl sera with DNA *in vitro* has prompted a study of the effect of antipurinoyl sera in biological systems. It has been demonstrated that antipurinoyl antibody inhibits the DNA-dependent transformation of pneumococci from streptomycin sensitivity to streptomycin resistance (1). Antipurinoyl sera have also been demonstrated to exert drastic alterations on the development of fertilized sea-urchin eggs (38). The effect of these antisera on other biological systems is under investigation.

SUMMARY

The specificity of the reaction of antipurin-6-oyl sera with thermally denatured DNA has been studied by means of hapten inhibition techniques. The relative order of effectiveness of various haptens as inhibitors of the complement fixation reaction between DNA and antipurin-6-oyl serum was found to be comparable to their relative order of effectiveness as inhibitors of the precipitin reaction between purin-6-oyl-protein conjugates and antipurin-6-oyl serum. Antipurin-6-oyl antibody has been purified and has been shown to be capable of reacting with thermally denatured DNA. It is concluded that the reactivity of these purine-specific antisera with DNA is a property of antibody with specificity for the purin-6-oyl moiety.

In addition, the reaction between antipurinoyl sera and DNA has been demonstrated by the techniques of radioimmunoelectrophoresis and passive cutaneous anaphylaxis.

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