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8. Normal medium: 28 m*M* NaCl; 28 m*M* disodium fumarate; 5 m*M* $CaCl_2$; 7 m*M* magnesium succinate; 2 m*M* KCl; 80 m*M* glutamine; 5 m*M* *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES); *p*H 7.4 with NaOH. Medium free of Ca and Mg: 38 m*M* NaCl; 28 m*M* disodium fumarate; 7 m*M* disodium succinate; 2 m*M* KCl; 80 m*M* glutamine; 5 m*M* TES; *p*H 7.4 with NaOH. Calcium- and magnesium-free Li medium: 38 m*M* LiCl; 38 m*M* dilithium succinate; 2 m*M* KCl; 80 m*M* glutamine; 5 m*M* TES; *p*H 7.4 with LiOH. DNP medium: normal medium plus 10^{-4}*M* dinitrophenol.
9. EDTA is used as saturated solution of disodium, or 3*M* solution of tripotassium, salt of ethylenediaminetetraacetic acid. EGTA is used as a saturated solution of ethylene-bis(oxyethylenenitrilo)tetraacetate.
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13. I thank Prof. W. R. Loewenstein and Dr. S. J. Socolar for interest and valuable discussions. Supported by PHS and NSF research grants. B.R. is on leave from the University of Munich.

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Double-Helical Polynucleotides: Immunochemical Recognition of Differing Conformations

Abstract. *Rabbit antibodies to double-helical RNA react by complement fixation with synthetic or natural double-strand RNA but not with native DNA. In turn, human (from systemic lupus erythematosus patients) antibodies to native DNA do not react with double-strand RNA. Both types of antibodies show cross-reactions (from 1 percent to 50 percent) with RNA-DNA hybrids, but antibodies to the hybrids do not react at all with double-strand RNA or with native DNA. Antibodies to polydeoxyguanylate·polydeoxycytidylate also failed to react with native DNA.*

Serums from rabbits immunized with complexes of methylated bovine serum albumin (MBSA) and the double-strand polyribonucleotide copolymer poly A·poly U (*1*) reacted, by precipitation and complement fixation, with either synthetic or naturally occurring double-strand RNA (*2*); they cross-reacted to a variable extent with RNA-DNA hybrid molecules, but did not react with either single-strand or double-strand DNA. This type of serum, used at a suitable dilution in quantitative complement fixation experiments, was an effective reagent for studying the small amount of double-strand RNA in the total RNA extracted from arbovirus-infected mammalian cells (*3*). Similar serums have been prepared by Lacour *et al.* (*4*) and by Plescia *et al.* (*5*), although the former group found a wider reactivity in some serum samples.

I now report some immunochemical relationships among a wider variety of double-helical polynucleotides. For this purpose, in an attempt to obtain antibodies that would react with native DNA, I injected rabbits with MBSA complexes of the synthetic hybrid poly A·poly dT (*6*), of the polydeoxyribonucleotide copolymers poly dG·poly dC, poly dAT·poly dAT, and of calf thymus DNA. Histone-DNA complexes were also used as immunogens. In a given course of immunization, animals were given two intradermal injections of complex (in complete Freund's adjuvant) a week apart and one intravenous injection a week after the second intradermal dose; the rabbits were bled a week after the intravenous injection. Each dose contained 30 to 50 μg of synthetic polymer or 50 to 200 μg of native DNA.

Of the polynucleotides used, the poly A·poly dT and poly dG·poly dC elicited specific antibody after a single course of immunization. The antiserums that they induced were compared with antiserum to poly A·poly U and antiserum to poly I·poly C (*7*), and with serums from patients with SLE, which react with native DNA (*8*). The complexes containing native DNA or poly dAT·poly dAT did not elicit specific antibody to the nucleic acid, in five and two animals, respectively, that received immunizing injections for as long as 6 months.

The immunochemical reactivities of the antiserums were selective for double-strand RNA, RNA-DNA hybrids, and double-strand DNA. At suitable dilutions, serums reacted by complement fixation with only one class of polynucleotide (Fig. 1). Although poly dG·poly dC reacted effectively with the

Table 1. Cross-reactions of antibodies to double-helical polynucleotides. Each serum was tested at varying dilutions with each antigen. The maximal complement fixation was plotted as a function of serum dilution and the dilution required for 50 percent maximal complement fixation was read by interpolation. Blank spaces indicate that there was no complement fixation at any dilution tested, the lowest dilution being 1/50.

Serum	Immunizing copolymer	Serum dilution required for 50 percent maximal complement fixation with:							
		Poly A·poly U	Poly I·poly C	Reovirus RNA	Poly A·poly dT	Poly dG·poly dC	Poly dA·poly dT	Poly dAT·poly dAT	Calf DNA
					Rabbit antiserums				
170	A·U	16,000	2,800	2,500	720				
171	A·U	12,000	2,000	850	< 50				
172	A·U	13,000	575	500					
201	A·U	7,000	1,550	2,400	< 50				
S-179	I·C	910	940	450	450				
S-180	I·C	480	580	370	300				
217	A·dT				10,000				
228	dG·dC					250			
229	dG·dC					2,000			
					Human SLE patients' serums				
SLE L					50	170	225	180	260
SLE R					350	800	1,200	1,000	1,450
SLE P					75	220	160	160	300
SLE E					45	60	85	85	85

antibodies to DNA in SLE serums, the antiserum to poly dG • poly dC was specific for the immunizing copolymer.

After being heated, poly A • poly U, reovirus RNA, poly A • poly dT, and poly dG • poly dC all lost complement-fixing reactivity with their respective antiserums, and in each case the temperature profile of the loss of reactivity showed a sharp transition with the expected melting temperature for the polynucleotide (Fig. 2). Formaldehyde (3 percent) was present in the heating mixture to prevent reannealing of the polymers. Thus, the complement fixation reactions involved the double-strand polynucleotides rather than contaminants in the preparations. The characterization of antibodies to native DNA in SLE serum has been described (*8*).

Table 1 presents a summary of quantitative studies of cross-reactions, in terms of the serum dilutions required to give a curve with a maximum of 50 percent complement fixation with a given antigen. Antiserums to double-strand RNA (either antiserums to poly A • poly U or antiserums to poly I • poly C) showed varying degrees of preference for the homologous antigen but reacted strongly with any of the double-strand RNA forms and not at all with any purely DNA form. The greater reactivity of antiserums to poly A • poly U with homologous antigen than with reovirus RNA or poly I • poly C may be due to the presence of some antibody to the triple-strand poly A • 2 poly U in the homologous system (*4*). Antibodies to native DNA in SLE serums reacted with any of the double-helical polydeoxyribonucleotides, but not at all with the purely RNA forms. Some SLE serums have been shown to contain separate antibodies to double-strand RNA (*9*), but these are often of low titer in complement fixation and were not detectable in the serums used in my study. Both the rabbit antibody to double-strand RNA and the antibody to native DNA in SLE serums showed cross reactions with RNA-DNA hybrids, to an extent varying from less than 1 percent for some serums to 50 percent for others.

The antiserum to hybrids did not react with either single-strand or double-strand forms of DNA or RNA. It did react to the same extent with either the synthetic hybrid or with the hybrid prepared by annealing single strands of T4 phage DNA with RNA from phage-infected *Escherichia coli* (*10*). The antiserum to poly dG • poly dC was specific even at a dilution of 1 to 50, though it still reacted with this polymer at 1 to 2000. It failed to react with either native or denatured DNA.

Physical measurements have shown that double-strand DNA and RNA differ in several aspects of conformation (*11*). These structural differences, which may appear to be minor, are reflected in complete and reciprocal lack of immunochemical cross-reactivity. Hybrid molecules are also differentiated, by variable and often low cross-reactivity with antiserums to double-strand RNA or DNA, but

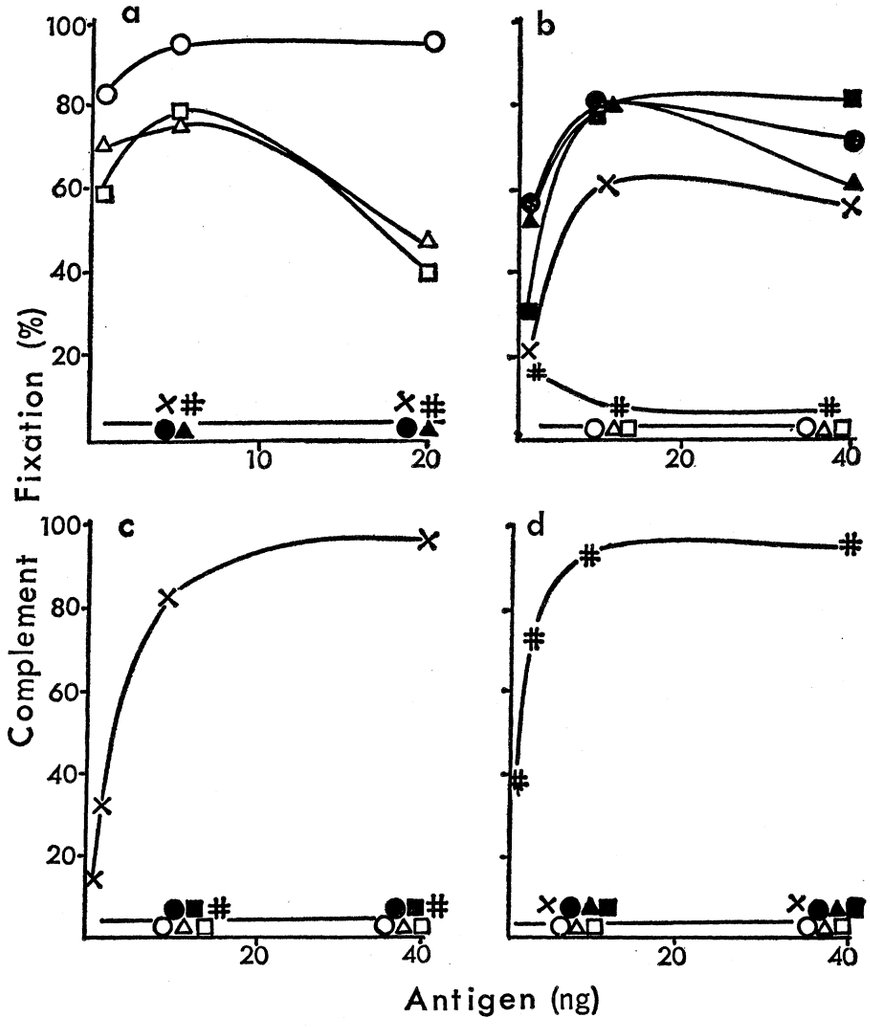

Fig. 1 (left). Complement fixation reactions of (a) rabbit antiserum to poly A • Poly U (1/1500); (b) human SLE serum, which reacts with DNA (patient L, Table 1) (1/110); (c) rabbit antiserum to poly dG • poly dC 229 (1/200); and (d) rabbit antiserum to poly A • poly dT 217 (1/200). ○, Poly A • poly U; △, poly I • poly C; □, reovirus RNA; #, poly A • poly dT; X, poly dG • poly dC; ■, poly dA • poly dT; ▲, poly dAT • poly dAT; and ●, native calf thymus DNA.

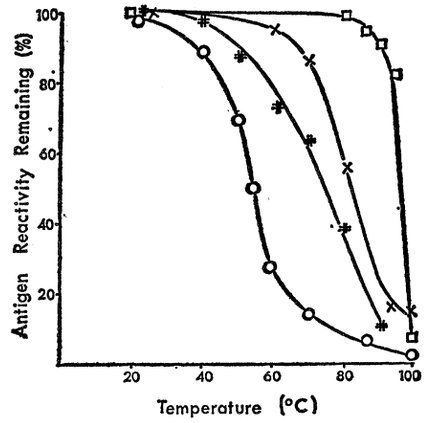

Fig. 2. Immunochemical measurement of the thermal denaturation of poly A • poly U (○) and reovirus RNA (□), measured with antiserum to poly A • poly U 170; and of poly A • poly dT (#) and poly dG • poly dC (X), measured with corresponding rabbit serums 217 and 229. The poly A • poly U and poly A • poly dT were heated in a buffer of 0.14*M* NaCl, 0.01*M* tris, *p*H 7.4; the reovirus RNA and poly dG • poly dC were heated in 0.014*M* NaCl, 0.001*M* tris *p*H 7.4 buffer.

especially by the narrow specificity of the antiserum to hybrids. Similarly, though poly dG • poly dC was like DNA in its reactions with SLE serums, it does differ from DNA in its x-ray diffraction pattern (*11*), and its corresponding antiserum distinguishes it completely from DNA. This antiserum to poly dG • poly dC did not react with even the DNA of *Pseudomonas fluorescens*, which has a 72 percent content of dG • dC base pairs. Thus the high content of dG • dC base pairs does not appear to confer the immunospecific reactivity of poly dG • poly dC to that DNA molecule.

The patterns of cross-reactivity of these serums support the conclusion that immunochemical specificity depends on the conformations of the double helices rather than simply on reactions with a given base or the presence of either ribose or deoxyribose as the carbohydrate component.

Antibodies to denatured DNA can be readily induced in animals by several methods (*12*), but there are no unequivocal examples of experimentally induced antibodies having selective reactivity with native DNA. The only sources of such antibodies remain the serums of patients with SLE or serums of NZB/W mice which have a disease very like, or identical to, SLE (*13*).

From our experience it appears that there is a very specific tolerance, in the rabbit, to the native DNA conformation and perhaps to such molecules as poly dAT • poly dAT, which closely resemble DNA in structure (*11*). When other kinds of related polymers are used as immunogens, only those conformational features that are quite distinct from native DNA are recognized, as indicated by the finding that none of the antibodies to these double-helical molecules reacted with DNA. On the other hand, antibodies from patients with SLE do appear to recognize more general features of the DNA molecule that are also present in many synthetic polydeoxyribonucleotides; this may be the result of a breakdown of the usual tolerance. Because of the low degree or lack of cross-reactivity among the classes of polymer, some antiserums are useful as specific reagents for detecting either double-strand RNA or RNA-DNA hybrid molecules.

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References and Notes

1. Abbreviations used are: poly A, polyadenylate; poly U, polyuridylate; poly I, polyinosinate; poly C, polycytidylate; poly dA, polydeoxyadenylate; poly dT, polythymidylate; poly dG, polydeoxyguanylate; poly dC, polydeoxycytidylate; poly dAT, a single-strand polymer with alternating polydeoxyadenylate and polythymidylate. Double-strand copolymers are represented by the combination of two single strands separated by a center dot (for example, poly A · poly U). SLE, systemic lupus erythematosus; MBSA, methylated bovine serum albumin.
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Chemical Basis of Hashish Activity

Abstract. *A sample of hashish was extracted consecutively with petroleum ether, benzene, and methanol. When tested intravenously in monkeys only the petroleum-ether fraction was active. This material was further fractionated. The only active compound isolated was Δ^1-tetrahydrocannabinol. Cannabinol, cannabidiol, cannabichromene, cannabigerol, and cannabicyclol when administered together with Δ^1-tetrahydrocannabinol do not cause a change in the activity of the latter, under the experimental conditions used. These results provide evidence that, except for Δ^1-tetrahydrocannabinol, no other major, psychotomimetically active compounds are present in hashish.*

The active Δ^1-tetrahydrocannabinol (Δ^1THC) (*1*) and $\Delta^{1(6)}$THC (*2*) have been isolated from *Cannabis sativa* preparations. The former is considered the predominant active component (*3–5*). Both THC's have been found to reproduce *Cannabis* activity in humans (*6*) and animals (*7, 8*). However, a systematic fractionation of *Cannabis* samples monitored by biological testing has not yet been reported. Hence, doubt has been expressed (*9*) as to whether Δ^1THC and $\Delta^{1(6)}$THC are the only active constituents and can replace crude marihuana or its extracts in *Cannabis* research. This is a point of importance. The use of chemically undefined and variable materials such as marihuana or hashish or their extracts has inherent methodological disadvantages. Reproducible biochemical, pharmacological, and clinical experimentation could be considerably facilitated by the possible use of properly characterized compounds instead of crude mixtures.

We now report some observations which indicate that Δ^1THC is indeed the only major active constituent in hashish. $\Delta^{1(6)}$THC, if present, did not represent more than 1 percent of the amount of Δ^1THC in the sample that we investigated.

A sample of hashish (502 g) of Lebanese origin, about 18 months old, was extracted eight times with a total of 4 liters of petroleum ether (b.p. 60° to 80°C) at 22°C. The extract (162 g) contained 23.1 g of Δ^1THC as determined by gas-liquid chromatography (*10*). The residue was extracted twice with boiling benzene (total solvent volume, 1500 ml). The extract (22.5 g) contained no Δ^1THC (*10*) and was inactive (*11*). The residue remaining after the benzene treatment was extracted twice with 1 liter of boiling methanol. The extract (19.1 g) which contained no Δ^1THC (*10*) was also inactive (*11*).

The petroleum ether extract was administered in doses containing 250 μg and 500 μg of Δ^1THC per kilogram (as determined by gas-liquid chromatog-