differences in patterns, while the nonallelic antigens A, B, and D were characterized by distinctly different fingerprints. It is concluded that the process of transformation within a strain from one specific serotype to another involves the *de novo* synthesis of a new sequentially different antigen in place of the old one.

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*THE ANTIGENIC DETERMINANTS OF DENATURED DNA REACTIVE WITH LUPUS ERYTHEMATOSUS SERUM**

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While it has long been known that sera of some patients with systemic lupus erythematosus contain antibodies reactive with deoxyribonucleic acid (DNA), our previous studies have shown that certain patients' sera react primarily with denatured DNA.[1] Evidence suggested that the antibodies varied, from serum to serum, in the size or specificity of the receptor sites.[2] With a given serum, DNA samples from varying sources reacted differently, as measured by quantitative complement

(C′) fixation. In studies with one lupus serum (J.W.), it was not possible to correlate these differences with over-all purine and pyrimidine base composition or with several physical chemical characteristics of the DNA samples.[2] Further studies with two other sera have been carried out, and the chemical nature of the DNA determinants required for reaction with these sera has been determined.

Materials and Methods.—Deoxyribonucleic acids: Salmon sperm DNA (California Corp. for Biochem. Res.) was further purified by the procedure of Marmur.[3] *Bacillus natto* DNA was also prepared by this method.[3] The preparation of bacteriophage DNA was described previously.[1] Bacterial DNA samples were supplied by J. Marmur. Denaturation of DNA was achieved by boiling, rapid chilling, and dilution of DNA samples, as described previously.[1] Samples with high guanine plus cytosine contents were boiled in dilute veronal buffer (0.015 *M*, pH 7.4).

Lupus erythematosus serum: Serum from *M.M.* was obtained through the cooperation of E. Chapman and J. Mills, Massachusetts General Hospital. Serum *In.* was obtained by R. Schwartz, New England Medical Center.

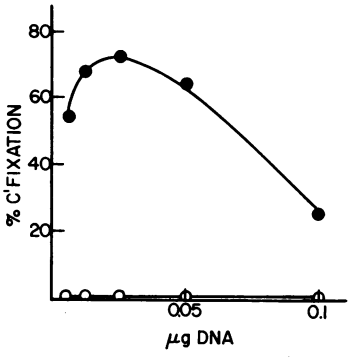

FIG. 1.—C′ fixation by lupus serum *M.M.* and *B. natto* DNA, native (○) and denatured (●). Serum dilution, 1–350.

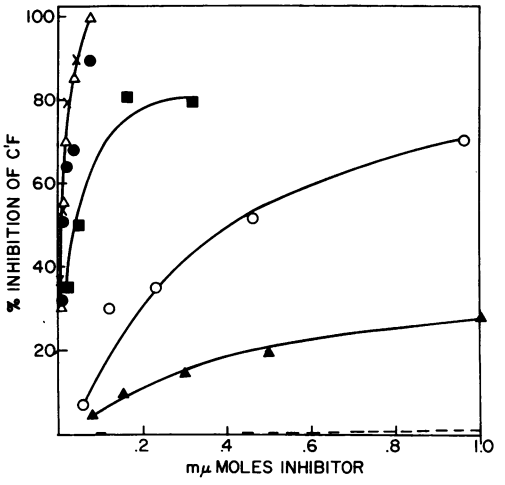

FIG. 2.—C′F inhibition of serum *M.M.* and denatured *B. natto* DNA by increments of mono- (—), di- (▲), tri- (○), tetra- (■), penta- (△), hexa- (●), and hepta- (×) pyrimidine nucleotide mixtures.

Polydeoxyribose adenine-thymine copolymer (Poly dAT) was obtained from L. Grossman. Polyuridylic acid (Poly U) and ribosomal ribonucleic acid (RNA) were given us by J. Marmur. For denaturation, poly dAT was boiled with formaldehyde (L. Grossman, personal communication).

Preparation of pyrimidine oligonucleotide mixture:[4] To 250 mg of salmon sperm DNA dissolved in 150 ml of distilled water was added 300 ml of a 3% solution of diphenylamine in 66% formic acid. The reaction mixture was allowed to stand at room temperature for 48 hr. Colored products and formic acid were removed by extensive ether extraction. The resulting aqueous mixture of free purines, inorganic phosphate (27% of total P), and oligopyrimidines was adjusted to pH 8.6 with ammonium bicarbonate-ammonium hydroxide buffer, and diluted to make the buffer concentration 0.01 *M*.

Phosphorus determinations: Total and inorganic phosphorus was determined according to the method of Chen, Toribara, and Warner,[5] in a total volume of 3.0 ml. Terminal phosphate was released by incubation with prostatic acid monophosphatase, in sodium acetate buffer, 0.1 *M*, pH 5.5, at 37°C overnight, and then determined as inorganic phosphorus. The acid monophosphatase was a gift of G. Schmidt.

DEAE cellulose chromatography: DEAE cellulose #40 (Brown Co.) was washed successively

with distilled water, 1 N NaOH, water, 95% ethanol, water, 95% ethanol, water, 1 N NaOH, 1 N $(NH_4)_2CO_3$, pH 8.6, and several times with 0.01 M $(NH_4)_2CO_3$, pH 8.6. The resin was packed in a column, 4 cm × 20 cm and washed 24–36 hr with the 0.01 M buffer. The sample was applied and the column was again washed with the 0.01 M buffer until all the purines were eluted, and then with 0.05 M buffer. Linear gradients from 0.05 M to 0.1 M to 0.15 M to 0.20 M to 0.275 M to 0.35 M to 0.50 M were applied. Fifteen ml fractions were collected at a flow rate of 2 ml per min, and the ultraviolet absorbance of each fraction was measured.

Paper chromatography: Oligonucleotides were separated by one- or two-dimensional paper chromatography, on Whatman #1 paper. Solvent I was isopropanol, 170; conc HCl, 44; H_2O to 250 ml. Solvent II contained t-butanol, 110; conc HCl, 10; H_2O, 30. Nucleotides, located by examination of the papers with a short-wave ultraviolet lamp, were eluted with 0.03 N HCl and their spectra determined with a Cary recording spectrophotometer. Appropriate paper blanks were included. Relative contents of thymine and cytosine were determined from the λ_{max} and ratio of O.D. 280/260, as compared with known mixtures.

Complement (C′) fixation: All C′ fixation experiments were performed as described elsewhere.[6]

Experimental and Results.—In direct C′-fixation experiments, serum $M.M.$ was found to react with thermally denatured DNA at a serum dilution giving no reaction with native DNA (Fig. 1). Different amounts of C′ fixation were observed in reactions with DNA samples from various sources. T_4 bacteriophage DNA, in which all cytosine is replaced by glucosylated hydroxymethylcytosine,[7] reacted with this serum. Hydroxymethylation of amino groups by denaturation in the presence of formaldehyde increased reactivity slightly.[8] No C′ fixation was observed with ribonucleic acid (RNA), denatured synthetic poly dAT, or poly U, which also failed to inhibit the reaction.

Complement fixation by serum $M.M.$ and denatured DNA was completely inhibited by equally small amounts of apurinic acid[9] and oligonucleotides from pancreatic deoxyribonuclease digestion of DNA. A mixture of polypyrimidine oligonucleotides of known general structure, $p(Py\text{-}dR\text{-}p)_n$,[4] was also an effective inhibitor. This mixture was applied to the DEAE-cellulose column, and seven well-separated peaks of polypyrimidine oligonucleotides were eluted. The ratio of total to terminal phosphorus indicated that the successive peaks consisted of oligonucleotides of increasing size, as has been previously reported.[4, 10] Material within

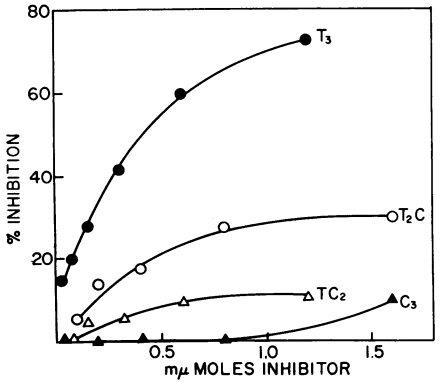

FIG. 3.—C′F inhibition of serum $M.M.$ and denatured *B. natto* DNA by increments of pyrimidine trinucleotides.

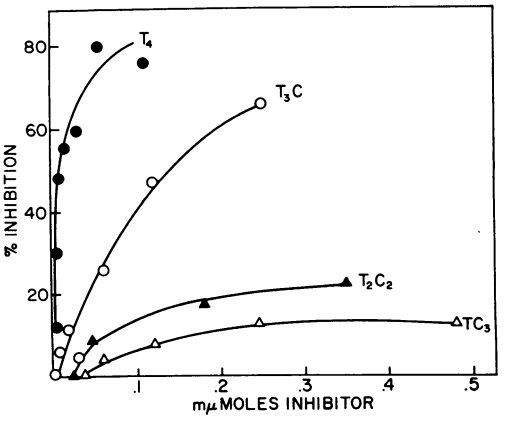

FIG. 4.—C′F inhibition of serum $M.M.$ and denatured *B. natto* DNA by increments of pyrimidine tetranucleotides.

each peak was pooled, evaporated to dryness, freed of the volatile salt by sublimation, and redissolved in 1.0 ml of water.

In seeking the most effective inhibitor of C′ fixation, it was thought that the size and perhaps the chemical nature of the reactive group of DNA might be determined in a manner similar to that which elucidated the antigenic determinants of the dextrans.[11]

Each fraction, which is a mixture of oligonucleotides of constant chain length but differing in the sequence and content of the cytidylic (Cp) and thymidylic (Tp) acid moieties, was examined for its ability to inhibit C′ fixation by serum *M.M.* and denatured DNA. The effectiveness of inhibition was found to increase with increasing size of oligonucleotide, up to the pentanucleotides, as shown in Figure 2. These oligonucleotides did not inhibit nonrelated immune systems such as the rabbit anti-T_4 bacteriophage DNA system, the determinant for which is glucosylated hydroxymethylcytosine.[12] It appeared that the chemical determinant might be about the size of a pentanucleotide, either a polydeoxyribose unit, or a given sequence of pyrimidine bases. Material from each chromatographic peak was separated by paper chromatography into its constituent components, varying in Cp and Tp content. The trinucleotides, T_{3p4}, T_2C_{p4}, TC_{2p4}, and C_{3p4}, were separated and tested for inhibition of the C′-fixation reaction. As shown in Figure 3, the trithymidylic acid was much more effective than any of the cytosine-containing trinucleotides. Similarly, tetrathymidylic acid was more effective than any of the tetranucleotides containing cytosine (Fig. 4). To obtain pentathymidylic acid, T_4 bacteriophage DNA was treated in the same way as the salmon sperm DNA, which did not contain this sequence. The progressively increasing inhibition by polythymidylic acids of increasing size up to a pentanucleotide is illustrated in Figure 5. As polythymidylic acid tracts larger than this do not occur in significant amounts in any DNA samples examined so far,[13] it was concluded that the determinant for DNA reactive with serum *M.M.* is a pentathymidylic acid. This would explain the relative lack of reactivity of native DNA, in which the bases are

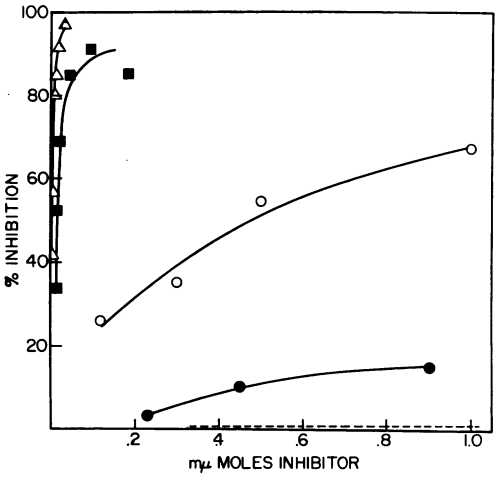

FIG. 5.—C′F inhibition of serum *M.M.* and denatured *B. natto* DNA by increments of T_{p2}(–), T_{2p3} (●), T_{3p4} (○), T_{4p5} (■), and T_{5p6} (Δ).

spatially not available. Such a conclusion is also consistent with the failure of RNA to react or to inhibit the reaction. Further, denatured poly dAT, in which adenine and thymine alternate so that no polythymidylic acid sequences occur, failed to react or inhibit. In formaldehyde-treated denatured DNA, which also reacts fully, thymine is not altered.

Serum *In.* also reacted with denatured DNA at a serum dilution giving no C′ fixation with native DNA. Inhibition of the reaction of this serum by polythymidylic acids of increasing size is shown in Figure 6. Effectiveness of inhibition increased up to the tetranucleotide, but was no greater with the pentanucleotide. As with serum *M.M.*, trithymidylic acid was a better inhibitor than cytosine-containing trinucleotides. It was concluded that the determinant for DNA reactive with serum *In.* is a tetrathymidylic acid.

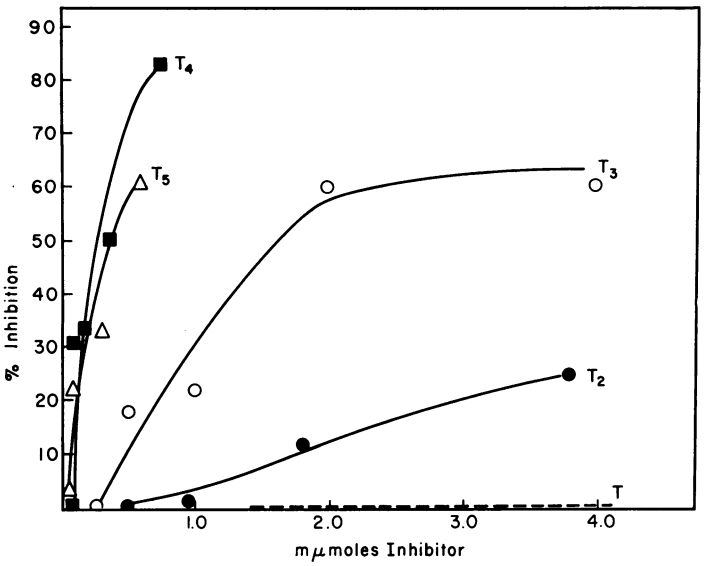

FIG. 6.—C′F inhibition of serum *In.* and denatured *B. natto* DNA by increments of T_{p_2} (–), T_{2p_3} (●), T_{3p_4} (○), T_{4p_5} (■), and T_{5p_6} (△). Serum dilution 1–200.

Discussion.—Inhibition of antigen-antibody reactions in immune systems of naturally occurring substances has been used to study the antigenic structure of dextrans,[11] blood group substances,[14] somatic antigens of the *Salmonella*,[15] streptococcal group-specific carbohydrates,[16] silk fibroin,[17] T-even bacteriophage DNA,[12] and to supplement structural studies of the pneumococcus type VI polysaccharide.[18] It has now been possible to identify the antigenic determinant of denatured DNA reacting in C′ fixation with antibodies in two lupus erythematosus sera. It should be re-emphasized[2] that possibly only the more avid portion of the total population of antibody molecules is being measured by the micro-C′-fixation technique because of the high dilutions of sera *M.M.* and *In.*, $^1/_{350}$ and $^1/_{200}$, respectively, used in the experiments. The measured population appears to be relatively homogeneous in size of receptor site; few molecules are completely tied up by dinucleotides or trinucleotides, as compared to tetra and pentanucleotides.

While the data shown in Figure 2 suggested that the antibody receptor site accommodates a determinant the size of a pentanucleotide, it remained to be learned whether the deoxyribose or the pyrimidine bases combined with the antibody. Since oligonucleotides of a given chain length all contain the same number of deoxyribose units but vary greatly in inhibitory effectiveness, it appears that the bases rather than the sugars are the reactive groups. Furthermore, denatured poly-dAT containing the deoxyribose units failed to inhibit. When trinucleotides and tetranucleotides of varying thymine and cytosine content were studied, it became clear that inhibitory effectiveness depended on thymine content; tetrathymidylic acid was twenty times as potent as the tetranucleotide with three thymines and one cytosine. The latter material was more effective than three thymine units alone, so that cytosine may contribute a small amount to the antigenicity, or sequences such as pTpTpCpTp may occur and react more effectively than pTpTpTp by virtue of the spatial arrangement of the three thymines.

The study of the pneumococcus type VI determinant[18] is in some ways analogous to the present work, in that a complex repeating unit with a terminal phosphate (galactoglucorhamnoribitol phosphate) was found to be a potent inhibitor. Removal of the phosphate of the S VI repeating unit slightly decreased inhibitory effectiveness. Similarly, removal of the terminal phosphates of tetrathymidine pentaphosphate (pTpTpTpTp) slightly reduced its inhibitory effectiveness with serum *M.M.*, but the resulting tetrathymidine triphosphate (TpTpTpT) was still much more potent than trithymidine tetraphosphate (pTpTpTp).

Interpretation of inhibition data with complex repeating units is complicated by the possibility that the inhibitor may be polyvalent (Kabat, personal communication). Though polyvalence of pentathymidylic acid cannot be excluded unequivocally, available data suggest that this inhibitor is, in fact, monovalent. No C′ fixation or inhibition was observed with an amount of denatured poly-dAT containing fifty times the weight of thymine present in an inhibitory quantity of pentathymidylic acid. If the determinant group were a monothymidylic acid, either C′ fixation or inhibition should have been observed. Furthermore, the reactive bases in the pentamer are separated by only a few Ångströms. It is thus difficult to visualize the attachment of several antibody molecules to this pentathymidylic acid. No direct C′ fixation was observed with pentathymidylic acid.

While sera *M.M.* and *In.* react with polythymidylic acid sequences, they differ somewhat. Antibodies in other lupus sera may also differ in specificity, and are being examined by inhibition techniques. Knowledge of the specificity of a given serum may allow it to be used as an analytical tool in the study of the structure of various DNA preparations. Speculative at present is the possibility that, if antibodies to DNA play any role in the pathogenesis of lupus erythematosus, the inhibitors used in these studies may eventually be of therapeutic value if they are able to neutralize the antibodies *in vivo*.

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*THE BIOSYNTHESIS OF RNA: PRIMING BY POLYRIBONUCLEOTIDES**

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Two distinctly different enzymes have been described as being involved in polyribonucleotide formation, polynucleotide phosphorylase[1] and RNA polymerase.[2–6] The former enzyme catalyzes the synthesis of RNA[7] in the presence of nucleoside diphosphates but in the absence of any known directing primer.[8] A similar reaction is catalyzed by RNA polymerase, but this enzyme requires the presence of all four nucleoside triphosphates as well as a DNA primer which directs the assembly of complementary RNA chains.[9, 10] Reports from a number of different laboratories have suggested that perhaps a third reaction, one which uses RNA as a primer for the synthesis of RNA, may also be present in living cells.[11–15]

In the course of testing various primers with somewhat crude preparations of RNA polymerase isolated from extracts of *Micrococcus lysodeikticus*, we observed that these preparations catalyzed the incorporation of labeled ribonucleotides into RNA if either DNA or plant viral RNA was present. Optimum labeling of RNA required the presence of all four triphosphates with either primer. While incorporation in the presence of viral RNA was relatively low (approximately 20% of the incorporation achieved with DNA) this phenomenon was consistently observed. However, as the *M. lysodeikticus* extracts were purified with respect to the DNA-dependent reaction (over 400 fold), the RNA-dependent incorporation was markedly reduced. This suggested that separate enzymes might be responsible for the priming of polyribonucleotide synthesis by DNA and RNA. Experiments with synthetic polynucleotides of known composition have provided a clear demonstration of the RNA-dependent synthesis of RNA in extracts from *M. lysodeikticus*, and are reported in this communication.