IV. DISCUSSION

The pathogenic significance of anti-DNA antibodies has been well documented in SLE and the antibody levels are claimed to correlate well with disease activity (Swaak et al., 1979). However, the qualitative and quantitative evaluation of anti-DNA antibodies is still being investigated for the perfection as reflected by the number of different methods that have been developed for their measurements. Radioimmunoassay (RIA) has been extensively used for the detection of anti-DNA antibodies (Talal and Pillarisetty, 1976). Sophisticated radioactive measuring instruments limit its use in laboratories where all these facilities are not available. These problems emphasize the need for a new simplified approach to measure anti-DNA antibodies.

The results obtained by spectrophotometric and fluorometric techniques are clearly indicative of their greater sensitivity than ID and CIE and comparability with PHA. Both the assays could therefore be employed for the detection of low affinity antibodies produced by immunized animals and SLE patients having low circulating concentrations of anti-DNA antibodies. Such antibodies practically act as univalent antibody and are not able to form stable precipitating complexes with antigen (Marghi et al., 1980). The reason for low incidence of anti-DNA antibodies by immunoprecipitation has not been established. Avidity, restricted
heterogeneity and the formation of soluble complexes are known factors which may effect the precipitin reaction.

High binding values were observed in normal human sera with dsDNA by spectrophotometric assay. The DNA binding of almost the same extent has been reported by other methods of measurements. This could be due to either low level of anti-DNA antibodies in normal human subjects or the nonspecific association of proteins and other materials found in serum with polyanionic DNA. Moreover, antibodies to nucleic acids have been found increased in aged healthy individuals (Schuller et al., 1981) which can further contribute to increased binding observed in apparently normal individuals. Both spectrophotometric and fluorometric methods described here are simple, specific, reproducible and suitable for use in clinical laboratories where facilities for radioimmunoassay do not exist.

SLE serum contain heterogeneous population of anti-DNA antibodies with varying degree of avidity. Some authors have reported that in humans circulating antibodies with high avidity for DNA are more likely to be associated with severe active disease and nephritis (Leon et al., 1977). Others have correlated low affinity circulating antibody with severe nephritis (Asano and Nakamoto, 1978). In mice, low avidity antibodies in the circulation appear at the time nephritis begins (Steward et al., 1975). Differences between avidity studies may reflect (i) different methods employed for the detection of anti-DNA antibodies
difficulty in calculating avidity with a bivalent antibody and a large relatively undefined DNA substrate and (iii) antibody heterogeneity (Hahn, 1982). In recent years, Farr assay and Millipore filter assays are under criticism for alleged inability to detect low avidity anti-DNA antibodies (Rubbin et al., 1978). Smeenk et al. (1982) reported the detection of low avidity antibodies in SLE serum by high sensitivity polyethylene glycol precipitation assay, not detected by conventional techniques.

Recently much interest has been directed in the use of ELISA in view of its operational simplicity and high sensitivity (Hendry and Herrmann, 1980; Aitkacic et al., 1981; Goodburn et al., 1981). Antibodies against native DNA have been evaluated by ELISA using glucose oxidase labelled antibodies (Labrouce et al., 1980). Their results were similar with those of Farr assay. ELISA has also been developed and evaluated for the detection of antibodies to DNA in experimental animals (Hillyer and Modeline, 1980). Recently ELISA for the measurement of anti-DNA levels has been reported (Kavai et al., 1982) using horse radish peroxidase-linked IgG, IgM and IgA conjugates. The substrate for monitoring the enzyme activity has generally been PNP-P (Voller et al., 1976; Harris et al., 1979).

In order to increase the sensitivity of ELISA, the conventional PNP-P has been replaced by a fluorescent substrate 4MU-P (Shalev et al., 1980). We adopted ELISA for the detection
and quantitation of anti-DNA antibodies using both PNP-P and 4MU-P as enzyme substrates. In the present study a 100 times lower concentration of the product (4MU) was detected by fluorometry in relation to PNP. This is not in full agreement with that of 1000 fold increased sensitivity of the fluorogenic substrate reported earlier (Shalev et al., 1980). This difference could possibly be accounted on the basis of different source of alkaline phosphatase used for preparing the conjugate resulting in lower specific activity. At different dilutions of antigen or antibody 9 times greater sensitivity was observed with fluorogenic substrate. However, at different conjugate dilutions the sensitivity of the assay was 16 fold higher with 4MU-P and are in full agreement to those reported by Shalev et al. (1980). We found an incidence of 57% of circulating DNA antibodies in SLE patients by ELISA with colorigenic and 71% positive with fluorogenic substrate. These results are clearly indicative of the greater sensitivity of fluorogenic substrate and could therefore be employed for the detection of low avidity antibodies. The same serum samples when tested by passive hemagglutination technique have an incidence of 36% for DNA antibodies. Some of the sera negative by PHA showed a strong positive reaction with ELISA. An agglutination inhibiting factor has been reported in thyrotoxic serum (Wilkin et al., 1979). It is possible that a similar factor is also present in some SLE sera inhibiting agglutination. The absence of hemagglutinating
antibodies in these sera could possibly be another contributory factor for low incidence.

We report the successful utilization of nylon as solid support for binding of DNA for use in ELISA in the detection of anti-DNA antibodies. High background noise poses major limitations in the success of enzyme immunoassays. Our experiments indicate that background activity can considerably be reduced by introducing nylon as solid support instead of conventional polystyrene surface. High back-ground values obtained due to nonspecific binding of the conjugate onto the walls of the test tube, have been reduced substantially in the present method by introducing last washing with distilled water and carrying out the enzymatic reaction with dried beads in separate tubes. ELISA in conjunction with nylon as solid support combines the advantages of (i) the high sensitivity obtained by the use of fluorogenic enzyme substrate (ii) low background values and (iii) facility for prolonged storage of antigen coated solid phase, making the assay highly sensitive, specific and economical. The ability of DNA coated nylon beads to retain antigenicity for a considerable period of time is of special importance since the cumbersome daily antigen coating procedure is avoided. The method is simple and could be conveniently employed for the detection of low avidity anti-DNA antibodies which were not detectable by commonly used techniques. It may allow an earlier diagnosis of the disease which might be helpful in the management of patients. The
possibility of distinguishing between different disease patterns is also feasible. Moreover, in the miniaturized form the method has scope in conditions where very small amounts of patients sera are available for assay.

The high incidence of anti-dsDNA antibodies detectable in patients with inactive SLE suggests that ELISA is potentially useful for diagnostic screening of asymptomatic individuals and patients with syndromes suggestive of SLE. The sensitivity of ELISA is comparable with that of radioimmunoassay and avoids the known dangers of isotope work. The ELISA test circumvents several problems inherent to radioimmunoassay procedure. Molecular weight of antigen does not influence the reaction of antibodies with DNA immobilized on solid phase. On contrary, in RIA only DNA molecules of molecular weight of 1x10^7 daltons or less are precipitated by a single antibody molecule, and therefore high molecular weight DNA requires a molar antibody-antigen ratio of greater than one for precipitation by ammonium sulfate (Aarden et al., 1976b). Antibody binding avidity is another factor which may account for differences between ELISA and RIA. ELISA directly measures gamma globulins thereby excluding interference by nonimmunoglobulin interactions with DNA such as C1q globulin and DNA binding proteins. In addition, the test system may be used to assay a variety of properties of the antibody like immunoglobulin class, complement fixing property and relative avidity. The ability to objectively quantitate antibody as well as the
potential for automation indicates that the solid phase immuno-
assay is potentially a useful procedure for the study of the
variety of antibodies occurring in the sera of patients with SLE.

Intramuscular injections of dsDNA and ssDNA complexed
with MBSA elicited antibodies against ssDNA in immunized rabbits
as detected by immunoprecipitation and hemagglutination techniques.
The comparison of immunological behaviour of DNA antibodies of
SLE sera and sera of immunized animals demonstrated the difference
in specificities of the two types of antibodies. Experimentally
induced antibodies reacted with ssDNA but not with dsDNA indica-
ting their specificity for bases and that the antigenic determi-
nants are not related to the backbone of nucleic acid. Earlier
studies in rabbits suggested that nucleic acids were haptens
(Plescia and Braun, 1967). Later work clearly demonstrated
that antibodies to double-stranded DNA could not be induced
whereas antibodies to double-stranded RNA, synthetic double-
stranded DNA, single-stranded DNA, oligonucleotides, nucleotides,
nucleosides and bases could easily be elicited (Stollar and
Ward, 1970). Anti-ss DNA appears frequently in diseases other
than lupus (Bell et al., 1975). These antibodies are frequently
induced inadvertently in humans, without appearance of anti-
native DNA antibodies, during treatment with procainamide and
certain other drugs which are known to induce antinuclear anti-
bodies. Many drugs have been implicated in the pathogenesis of
SLE-related syndromes which include procainamide, hydralazine,
sulfonamides, isoniazid, trimethadione, phenylbutazone, streptomycin, tetracycline and griseofulvin (Blomgren et al., 1972). It has been suggested that these drugs with the ability to form complexes with DNA and DNA-protein are most likely to induce antinuclear antibody formation (Grabar, 1974). Recently antinuclear antibodies have been reported in psoriatic patients undergoing photochemotherapy. Antibodies to dsDNA have not been detected although, it is known that psoralen forms a covalent photoadduct with DNA in the presence of UV light and makes its immunogenic (Zarebska et al., 1978). In these studies Farr assay was employed for the detection of such antibodies which has been shown to be unable to detect low avidity anti-DNA antibodies.

ELISA on nylon as solid support has been successfully utilized for the detection of antibodies against DNA, RNA, ssDNA and DNA-psoralen adduct in animals immunized with DNA-psoralen adduct. We report that on intercalation with psoralen in the presence of UV-A light, DNA becomes immunogenic and antibodies formed have reactivity against DNA-psoralen adduct and dsDNA. These antibodies showed poor crossreactivity with ssDNA and RNA indicating that the antibodies probably are conformational directed. Our results show that although it is not possible to immunize animals against dsDNA but when DNA is intercalated with psoralen, this apparently nonimmunogenic molecule become immunogenic. This might be as a result of some structural changes in the molecule induced by intercalation with psoralen. The low
reactivity of immunized sera with ssDNA and RNA could probably be due to nonavailability of the antigenic bases to elicit antibody response resulting in the failure of the appearance of anti-ssDNA specific antibodies.

SLE anti-DNA antibodies isolated by affinity chromatography using dsDNA-Sepharose 4B columns were used to study the specificity of immune sera. SLE antibodies reacted specifically with dsDNA and showed strong reactivity with DNA-psoralen adduct while single-stranded DNA and RNA exhibited low reactivity. The antigenic specificity of these antibodies was indistinguishable from antibodies raised by immunization of animals with DNA-psoralen adduct as is clear from the results obtained in ELISA.

The specific etiological factors that cause SLE have eluded identification to the present time with the exception of patients who have a lupus like syndrome in relation to exposure to certain drugs (Hess, 1982). Genetic, viral, hormonal and environmental factors are all probably involved in the pathogenesis of this disease providing evidence for a truly multifactorial etiology. In drug induced lupus it has been suggested that these agents interact with DNA-protein complex in vivo and the resulting interaction renders it immunogenic and result in the production of autoantibodies. The formation of covalent steroid-protein adducts on incubation of albumin with cortisol or 16 α-hydroxyestrone and its possible involvement in the patho-
genesis of SLE has recently been attempted (Bucala et al., 1982). On the basis of our observation that covalent DNA-psoralen adduct elicit anti-DNA antibodies in experimental animals, it could be assumed that similar structural changes in DNA by interaction with above mentioned agent(s), might render it highly antigenic in susceptible individuals initiating and contributing to the pathogenesis of SLE.