whether the thrombopoietic effect of plasma from such donors is the result of an increased concentration of a stimulating factor or decreased concentration of an inhibitory material.

Summary. Injection of plasma from rabbit donors made thrombocytopenic by Myleran or anti-platelet serum into 15 unstressed rabbit recipients raised platelet counts to 166% of control levels 4 days following transfer. These results are highly significant ($p<0.001$) when compared to the mild thrombocytopenia produced in 15 rabbits receiving plasma from normal donors or when compared to the daily variations in platelet levels of 9 uninjected rabbits. The response of normal and splenectomized recipients was similar. These results suggest that plasma contains stimulatory and inhibitory factors which regulate platelet levels.

I acknowledge with gratitude the advice and criticism offered by Dr. Marjorie B. Zucker, and technical assistance of Elaine Beck, Elizabeth Davidson, Myrtle Martin, Eva Neer and Ethel Spector.

1. Brecher, G., Schneiderman, M., Cronkite, E. P., *Am. J. Clin. Path.,* 1953, v23, 15.
2. Odell, T. T., Jr., Tausche, F. G., Gude, W. D., *Am. J. Physiol.,* 1955, v180, 491.
3. Aas, K. A., Gardner, F. H., *J. Clin. Invest.,* 1958, v37, 1257.
4. Adelson, E., Rheingold, J. J., Crosby, W. H., *J. Lab. Clin. Med.,* 1957, v50, 570.
5. Leeksma, C. H. W., Cohen, J. A., *Nature,* 1955, v175, 552.
6. Zucker, M. B., Ley, A. B., Mayer, K., *J. Lab. Clin. Med.,* 1961, v58, 405.
7. Craddock, C. G., Adams, W. S., Perry, S., Lawrence, J. S., *J. Lab. Clin. Med.,* 1955, v45, 906.
8. Matter, M., Hartmann, J. R., Kautz, J., DeMarsh, B. Q., Finch, C. A., *Blood,* 1960, v15, 174.
9. Brecher, G., Cronkite, E. P., *J. Appl. Physiol.,* 1950, v3, 365.
10. Elliott, R. H. E., Whipple, M., *J. Lab. Clin. Med.,* 1940, v26, 489.
11. Keleman, E., Lehoczky, D., Perkedy, J., Oserhati, I., Rak, K., *Lancet,* 1960, v1, 1134.
12. Schulman, I., Pierce, M., Lukins, A., Currimbhoy, J., *Blood,* 1960, v16, 943.

Received May 15, 1961. P.S.E.B.M., 1961, v108.

Comparative Reactivities of Non-fibrous and Fibrous Forms of Desoxyribonucleoprotein with Lupus Erythematosus Serum.* (26875)

THOMAS S. EDGINGTON AND ROY L. WALFORD (Introduced by Sidney C. Madden)
Department of Pathology, University of California Medical School, Los Angeles

Numerous investigators have reported that factors in the sera of patients with disseminated lupus erythematosus (LE) may react with whole cells(1), isolated nuclei(2,3), desoxyribonucleic acid (DNA)(3,4,5), desoxyribonucleoprotein (DNP)(4,6,7,8), and histone(9). It has been demonstrated that there are separate components of LE sera that react with each of the "antigens". This reactivity has been demonstrated by a variety of immunologic technics including complement fixation, latex agglutination, hemagglutination, precipitation, and fluorescence microscopy. The so-called "LE factor" is reactive only with DNP and not with isolated histone or DNA(8). In detecting the anti-DNP activity of LE sera, fibrous preparations of DNP have been utilized. These forms of DNP dissociate into DNA and histone in the molar NaCl utilized in preparation, and it is not certain that the re-association product has the identical spatial association of histone and DNA present in DNP *in vivo*. Among the numerous preparations of DNP described in the literature, only one does not dissociate in molar salt solution(10, 11). No immunologic investigations are on record, so far as we are aware, utilizing non-fibrous DNP in the role of antigenic component. The purpose of the present investigation was to determine the reactivities of non-fibrous and fibrous DNP with LE sera

* This study was aided by research grant from Nat. Inst. Health.

by means of the complement fixation test. It was found that the non-fibrous DNP showed a much greater reactivity under the conditions of our experiments.

Materials and methods. Serum specimens were obtained from 14 patients with proven systemic lupus erythematosus. Each patient had shown a positive LE test on the day that the serum specimen was obtained. The specimens were stored at −56°C until used.

Fibrous DNP was prepared by the method of Rotherham *et al.*(12), and non-fibrous DNP by the technic of Luck *et al.*(10). In each case rat liver was employed as the original tissue. Several comments will be made about the preparation of non-fibrous DNP because it is easily converted to the fibrous form by minor variations in the preparative procedure. Non-fibrous DNP is prepared as follows. In a cold room (0-4°C) a suspension of rat liver is made in 0.002 M citric acid. This is homogenized in a low-speed Waring blendor at pH 6.1. After cellular rupture is achieved (about 3 minutes with the Waring blendor running on 45 volts) the suspension is filtered through cheese cloth and centrifuged. The supernatant and mitochondrial layers are decanted, and the nuclear sediment is washed 3 times with 0.02 M sodium acetate buffer at pH 6.1. The washed nuclei are then extracted for one hour with 0.14 M NaCl containing 0.02 M sodium citrate-citric acid at pH 6.5. After ultracentrifugation at 20,000 × g for 15 minutes the residual sediment is suspended in 1 M NaCl containing 0.02 M sodium citrate at pH 8.3. After one hour the suspension is centrifuged at 26,000 × g for 15 minutes. The supernatant is recovered and diluted with 6 volumes of distilled water, thereby precipitating the non-fibrous DNP from solution as a fine white particulate sediment. The non-fibrous DNP is redissolved in 1 M NaCl, ultracentrifuged, and precipitated as above. The DNP is suspended in 25 ml water and dialyzed overnight at 1°C against 4 liters of distilled water. The dialyzed solution is centrifuged at 26,000 × g and the supernatant is frozen in aliquots for later use. The critical points in the preparation are initial homogenization and washing of the nuclei. Homogenization must be only sufficiently long and vigorous to insure maximal cellular rupture with minimal nuclear trauma. The homogenate should be maintained at pH 6.1 ± 0.1. If the subsequent washing with acetate buffer is inadequate cytoplasmic residue will remain as a contaminant. Fibrous DNP was obtained in a number of preparations, possibly due to inadequate control at these points. In a few preparations non-fibrous DNP was noted to change to the fibrous form after precipitation of the DNP from 1 M NaCl.

The purified non-fibrous and fibrous forms of DNP, prepared as above, were made up in 1 M NaCl solutions to a concentration containing 36 μg DNA per ml, as determined by the diphenylamine reaction. Aliquots of each solution were then diluted with 1 M NaCl so that 0.1 ml would contain the equivalent of 4.5, 0.45, 0.045, and 0.0045 μg of DNA respectively (Table I). These amounts were used as "antigen" in the complement fixation test with LE serum.

The complement fixation test was performed as follows. One-tenth ml of each DNP dilution and 0.6 ml of distilled water were set up in a series of 10 × 75 mm test tubes. Two-tenths ml of LE serum (previously inactivated at 56°C for 30 minutes) was added to each tube. The tubes were left at room temperature for 20 minutes. One ml of complement (2 full units) was added and all tubes placed in the refrigerator for 16 hours, followed by 10 minutes incubation in a 37°C water bath. One-half ml of hemolysin (2 full units) was added to each tube, followed by 0.5 ml of a 2% suspension of sheep cells, and all tubes were thoroughly shaken. Simultaneous complement and serum blanks were run. After shaking, all tubes were placed in a 37°C water bath and were examined at 5-minute intervals for complete hemolysis of the complement blank. When this had occurred, the tubes were incubated 10 minutes more, then the degree of complement fixation was read as 0 to 4+, depending on whether approximately 100%, 75%, 50%, 25%, or no hemolysis was observed by comparison with appropriate controls.

TABLE I. Complement Fixation Reaction with LE Sera and Serial 10-Fold Dilutions of Non-Fibrous and Fibrous Desoxyribonucleoprotein.

	DNP concentration/tube (μg)							
	Fibrous DNP				Non-fibrous DNP			
Patient	4.5	.45	.045	.0045	4.5	.45	.045	.0045
Cd	2+	1+	0	0	2+	1+	0	0
Co	4+	1+	0	0	4+	3+	1+	0
Di	1+	0	0	0	4+	3+	0	0
Pm	4+	1+	0	0	4+	4+	3+	0
Lz	3+	0	0	0	4+	3+	1+	0
Rs	4+	1+	0	0	4+	3+	1+	0
Sv	3+	0	0	0	4+	3+	0	0

Results. Seven of the 14 LE sera demonstrated complement fixation with both forms of DNP under the conditions of these experiments (Table I). In general the reactive sera were from patients with strongly positive LE tests, and the negative specimens from patients with more weakly positive tests. A higher incidence of reactivity might have been obtained with stronger initial "antigen" concentrations of DNP, but this was not investigated. The non-fibrous DNP proved to fix complement better, in the presence of LE sera, than the fibrous DNP. This increase in reactivity lay between 10 and 100 times. There were on the other hand no obvious qualitative distinctions between the two forms of DNP in that the LE sera appeared to react with both forms of DNP or not at all.

Discussion. The present study demonstrates that non-fibrous DNP reacts with LE serum at a significantly lower concentration than the fibrous form, and thus appears to be a stronger "antigen" under these conditions. The significance of this is not clear. In general, "native" substances show stronger immunologic reactivity than their degraded or denatured counterparts; the fibrous DNP is clearly a dissociated form and possibly has been modified from its "native" state. The complex relationship between DNA and histone (13,14) may differ between the 2 forms of DNP and may have modified the spatial relationships of active sites responsible for the reaction with LE serum. However, any differences in molecular structure are apparently not great enough to abolish reactivity with LE serum. Does the anti-DNP component of LE serum arise in response to partially degenerated or altered cellular material occurring in the course of the disease If so, it might represent a secondary phenomenon. On the other hand it and similar factors may be concerned in the initiation of cellular damage. A strong reaction with a more "native" form of DNP would be consistent with the latter possibility. The question is, which form of DNP is the more "native"? This problem is further complicated by the absence of good criteria for identifying immunologically "native" DNP, and the fact that "altered" substances are sometimes more active than "native" substances(15).

Summary. The comparative reactivities of the non-fibrous and fibrous forms of desoxyribonucleoprotein were studied in the complement fixation test with LE sera. On the basis of "antigen" dilution experiments the non-fibrous DNP was shown to be between 10 and 100 times more reactive than fibrous DNP.

1. Walford, R. L., *Leukocyte Antigens and Antibodies,* Grune & Stratton, N. Y., 1960, Chap. V.

2. Holman, H. R., Kunkel, H. G., *Science,* 1957, v126, 162.

3. Robbins, W. C., Holman, H. R., Deicher, H., Kunkel, H. G., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 575.

4. Miescher, P., Strassle, R., *Vox Sanguinis,* 1957, v2, 283.

5. Pearson, C. M., Craddock, C. G., Simmonds, N. S., *J. Lab. and Clin. Med.,* 1958, v52, 580.

6. Christian, C. L., Meddez-Bryan, R., Larson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 820.

7. Miescher, P., *Vox Sanguinis,* 1957, v2, 145.

8. Holman, H., Deicher, H. R., *J. Clin. Invest.,* 1959, v38, 2059.

9. Bardawil, W., Toy, B., Galins, N., *Lancet,* 1958, v1, 888.

10. Luck, J. M., Kupke, D. W., Rhein, A., Hurd, M., *J .Biol. Chem.,* 1953, v205, 235.

11. Kupke, D. W., Eldredge, N. T., Luck, J. M., *ibid.,* 1954, v210, 295.

12. Rotherham, J., Schottelius, D. D., Irvin, J. L., Irvin, E. H., *ibid.,* 1956, v223, 817.

13. Zubay, G., Doty, P., *J. Mol. Biol.,* 1959, v1, 1.

14. Wilkins, M. H. F., *Biochem. Soc. Symp.,* 1957, v14, 13.

15. Pogell, B. M., McGilvery, R. W., *J. Biol. Chem.,* 1952, v197, 293.

Received March 6, 1961. P.S.E.B.M., 1961, v108.

Bacterial Flora of the Mouse Uterus.* (26876)

A. TREGIER AND F. HOMBURGER
Bio-Research Institute, Cambridge, Mass.

While it is usually assumed that the uterus, like most internal organs, is sterile in the absence of manifest disease, the studies here reported indicate that microorganisms are present in the uterine cavity of a certain percentage of mice which, on surgical examination and routine histologic study, reveal no uterine disorder. A similar prevalence of endouterine organisms exists in immature mice with closed vaginas, indicating that the infection is not an ascending one.

Methods. One thousand and eighty mice of the Swiss, C57BR/cd, C57BL/6 and BALB/c strains underwent laparotomy under nembutal anesthesia. The uterus was stretched out on the tips of a forceps and the cervix was ligated. Each horn was pierced by a 25-gauge needle attached to a tuberculin syringe containing sterile broth or Ringer's Solution. One to 2 drops were injected, reaspirated and inoculated into sterile broth. In some cases, a dilution of 1:1000 of the aspirate was placed on blood agar plates. The cultures were incubated for from 24 to 48 hours and the bacteria examined on gram stained smears. In addition, a separate group of 222 C57BR/cd uteri were cultured in broth and the bacteria present in 25 of the 48 infected cultures were identified by routine bacteriological methods.† Two to 3 months later, a second laparotomy was performed and, in the BALB/c, Swiss and C57BR/cd mice, the uterine fluid which had collected was aspirated, examined by Gram stains and cultured in broth. After each laparotomy, penicillin was sprinkled into the peritoneum and achromycin was given for 3 days in the drinking water‡. The prevalence of bacteria found on broth cultures of the uteri at the first and second examinations is shown in Table I.

On the blood agar plates, the most prevalent organisms were identified§ as *E. coli, B. aerogenes, Proteus vulgaris* and *Streptococcus fecalis,* and numbers of the cultures per plate varied from 8 to innumerable. In the 25 broth cultures from C57BR/cd mice, the following bacteria were identified: *Corynebacterium hoffmannii* in 68%, *Proteus vulgaris* in 16%, *Streptococcus fecalis* in 4%, *Staphylococcus albus* in 4%, *E. coli* in 4%, and mixed infections of *Proteus vulgaris* and *Corynebacterium hoffmannii* in 4%.

All of the uteri tested showed no anomaly on clinical examination. Histologic sections were prepared on 50 random uteri and in some of them Gram stains revealed the presence of bacteria in the mucoid material adhering to the endometrium.‖

The uterine fluid (Uterone) which accumulated after cervical ligation(1) in from 2 to

* Supported by grants from Nat. Inst. Health, Bethesda, Md. and Am. Cancer Soc., Inc., New York.

† The cooperation of Sumner M. Joress, of New England Center Hospital, is gratefully acknowledged.

‡ Achromycin was graciously provided by the Lederle Co., Division of American Cyanamid, Pearl River, N. Y.

§ The cooperation of Dr. E. Kass, of Boston City Hospital, is gratefully acknowledged.

‖ The histologic sections were prepared by John R. Baker, and interpreted by Dr. A. B. Russfield.