Blood Coagulation and the L. E. Cell Phenomenon

By Stanley L. Lee, Lawrence I. Schwartz, and Sanford Pariser

The L. E. cell phenomenon is observed as a peculiar alteration in the leukocytes of blood or bone marrow of patients with lupus erythematosus, induced by a constituent of the plasma or serum gamma globulin. It does not depend on any intrinsic abnormality of the leukocytes themselves and may be induced not only in leukocytes from the patient but in those of normal individuals and in the leukocytes of several mammalian and avian species.

Recently a relationship between the L. E. cell phenomenon and blood coagulation has become apparent. The L. E. cell phenomenon was first demonstrated by incubation of heparinized bone marrow. However, it was soon apparent that it also took place when coagulation was not inhibited. Later it was shown that the phenomenon occurred with much greater intensity when blood was allowed to clot spontaneously than when coagulation was inhibited by citrate, oxalate or heparin.

More recently Zimmer and Hargraves confirmed and enlarged upon this observation and concluded that anything which interferes with coagulation interferes with the L. E. cell phenomenon. Although no direct evidence was presented, the platelets were implicated as the significant part of the coagulation system related to the L. E. cell.

Studies on the specific component of the coagulation process necessary for the activity of the L. E. cell factor form the basis for this report.

Materials and Methods

Fifteen patients with systemic lupus erythematosus (from the wards and Out-Patient Department of The Mount Sinai Hospital) whose blood contained the L. E. cell factor formed the clinical material for this study.

The L. E. cell phenomenon was studied in leukocytes from human peripheral blood only, using either the patient's own leukocytes or cells from normal or other donors.

The degree of intensity of the L. E. cell phenomenon was estimated by methods and criteria described in a previous publication. All slides were interpreted by one observer, and insofar as possible, readings were "blind." Definite enhancement or inhibition (all or none) of the L. E. cell phenomenon was the determining factor in interpreting an experiment as decisive. Where finer shades of differentiation existed, the experiment was considered a failure.

Technics varied with the experiments and will be described in detail in connection with each experiment.

Results

1. Confirmation of relationship between coagulation and the L. E. cell phenomenon

A clean venepuncture was made; blood was withdrawn from a patient whose blood contained the L. E. cell factor, and was transferred immediately to tubes.
as indicated in table 1. All tubes were incubated at 37 C. for 60 minutes, centrifuged at 300 G for five minutes and the serum or plasma pipetted off. The remaining contents of each tube were briskly agitated 20-30 times with a wooden applicator stick. Smears were then made from small drops of this material and examined for the intensity of the L. E. cell phenomenon. Table 1 shows the results of one such experiment. Numerous similar experiments have yielded like findings.

Blood rendered incoagulable by any of the anticoagulants showed a striking reduction in the L. E. cell phenomenon as compared with blood permitted to clot spontaneously in glass tubes. Prolongation of coagulation by the use of siliconized glass tubes did not inhibit the L. E. cell phenomenon nor diminish it to any great extent.

### Table 1

<table>
<thead>
<tr>
<th>Tube</th>
<th>Contents</th>
<th>% PMN showing L.E. involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (siliconized)</td>
<td>0.1 cc 5% sequestrene-Na + 1 cc blood</td>
<td>less than 1</td>
</tr>
<tr>
<td>2 (not &quot;&quot;)</td>
<td>0.1 cc 5% sequestrene-Na + 1 cc blood</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>3 (&quot; &quot;&quot;)</td>
<td>0.1 cc 0.1M Sodium Citrate + 1 cc blood</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>4 (&quot; &quot;&quot;)</td>
<td>0.1 cc 0.1M Sodium Oxalate + 1 cc blood</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>5 (&quot; &quot;&quot;)</td>
<td>0.1 cc normal saline containing 1 mg heparin + 1 cc blood</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>6* (siliconized)</td>
<td>0.1 cc normal saline + 1 cc blood</td>
<td>5</td>
</tr>
<tr>
<td>7* (not &quot;&quot;)</td>
<td>0.1 cc normal saline + 1 cc blood</td>
<td>10</td>
</tr>
</tbody>
</table>

* clotted.

### Table 2

<table>
<thead>
<tr>
<th>L.E. plasma 0.5 cc (+ WBC + intact platelets + 0.1 M sodium citrate)</th>
<th>Intensity of L.E. cell phenomenon†</th>
</tr>
</thead>
<tbody>
<tr>
<td>+0.5 cc platelets (produced by high-speed centrifugation of normal platelet-rich plasma in glass tube)</td>
<td>Strongly positive</td>
</tr>
<tr>
<td>+0.5 cc supernatant from platelets</td>
<td>Almost or completely negative</td>
</tr>
<tr>
<td>+0.5 cc fresh normal serum (one hour old)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>+0.5 cc aged normal serum (7 days at 4° C.)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>+0.5 cc normal saline containing 200 NIH units of bovine thrombin (Parke-Davis)*</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>+0.5 cc normal saline</td>
<td>Strongly positive</td>
</tr>
<tr>
<td>+0.5 cc normal saline containing approx. 4 mg rabbit brain thromboplastin extract (Difco)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>+0.5 cc 0.02 M CaCl2*</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

* tube clotted.
† Since this table combines the results of several experiments, intensity has been expressed in general terms only.
2. Relation of coagulation factors to L. E. cell phenomenon

A group of experiments was performed in which various elements of the coagulation mechanism were tested individually for their effect on the L. E. cell phenomenon. In these experiments coagulation was inhibited and various coagulation factors were added to study this effect on the occurrence and intensity of the L. E. cell phenomenon. Blood was drawn through a clean venepuncture with siliconized equipment. It was transferred to iced siliconized tubes containing sodium citrate, which were refrigerated until the red cells had settled sufficiently to provide adequate amounts of plasma for the tests. (Since all the L. E. patients had rapid erythrocyte sedimentation rates, this period never exceeded one hour.) Aliquots of this platelet-rich, leukocyte-rich L. E. plasma were then added to previously prepared ingredients containing various clotting agents as shown in table 2 and incubated at 37 C. for one hour. At the end of an hour all tubes were centrifuged for one minute, and smears were made from the sediments and examined for the intensity of the L. E. cell phenomenon.

Table 2 summarizes the results of these experiments. Labile factor, S.P.C.A., thrombin and fibrin appear to play no role in the activation of the L. E. cell factor. A constituent derived from platelets is necessary for this activation to occur.

3. Relation of platelets to the L. E. cell phenomenon

That platelets are important in the reaction was also shown by the following experiment (table 3). Blood containing the L. E. cell factor was drawn by clean venepuncture into siliconized apparatus containing 0.1 M sodium citrate. One sample was centrifuged at 300 G for ten minutes and another at 3550 G for thirty minutes to produce platelet-rich and platelet-poor plasmas respectively, both free of white cells. To 0.5 cc. of each of the supernatant plasmas (in unsiliconized tubes) was added an equal quantity ofuffy coat from citrated blood of a patient with idiopathic thrombocytopenic purpura. After thorough mixing of each tube, platelet counts were made. 0.1 cc. of 0.1 M calcium chloride was then added to each tube. All clotted promptly. Tubes were incubated for one hour at 37 C. At the end of that time they were centrifuged briefly, serum was pipetted off, clots were agitated with applicator sticks and smears were made and examined for the L. E. cell phenomenon. As shown in table 3, in every instance greater intensity of L. E. cell phenomenon was observed in the tube with the greater concentration of platelets.

There thus appears to be a positive relationship between a factor or factors

<table>
<thead>
<tr>
<th>Patient</th>
<th>Platelet count (per cu. mm.)</th>
<th>%PMN involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>93,000</td>
<td>13</td>
</tr>
<tr>
<td>F</td>
<td>1,500</td>
<td>3</td>
</tr>
<tr>
<td>O</td>
<td>74,000</td>
<td>8</td>
</tr>
<tr>
<td>O</td>
<td>3,500</td>
<td>4</td>
</tr>
<tr>
<td>R</td>
<td>80,000</td>
<td>11</td>
</tr>
<tr>
<td>R</td>
<td>2,000</td>
<td>3</td>
</tr>
</tbody>
</table>
derived from platelets (as produced by high speed centrifugation in plasma or spontaneously as in clotting) and the intensity of the L. E. cell phenomenon. Experiments have shown that if platelets are maintained intact, (siliconized glassware, care in venepuncture, prompt cooling and choice of anticoagulant), the L. E. cell phenomenon may be completely or almost completely inhibited. Thus it seems that the intact platelet per se is not involved in the phenomenon but that a constituent released from platelets is necessary. Platelets are, of course, complex structures containing many different substances and factors. The best characterized of these is the platelet thromboplastic factor. Rabbit brain thromboplastin (Difco Laboratories), when added to plasma containing the L. E. cell factor, consistently enhanced the L. E. cell phenomenon produced by this plasma (table 2). Brain thromboplastin seemed at least as potent as added platelets in this regard.

**DISCUSSION**

Our early experiments, as well as those of Zimmer and Hargraves, showed that blood coagulation somehow leads to an accentuation of the L. E. cell phenomenon. The experiment summarized in table 1 confirms this fact. Accentuation after clotting suggested that a substance was elaborated during clotting which aided the production of the L. E. cell phenomenon. The experiments summarized in table 2 were intended to discriminate among the various coagulation factors. Addition of thrombin to citrated plasma failed to produce any accentuation of the L. E. cell phenomenon. This eliminated both thrombin and fibrin from further consideration. Neither fresh nor aged normal serum led to any increase in L. E. cell production; thus the accelerator factors could be eliminated. There remained under consideration the thromboplastic factors of plasma and the platelets. Platelets apparently supplied the material in question, since in their presence, and in the absence of other phenomena of clotting, marked accentuation of the L. E. cell phenomenon was observed.

The experiments shown in table 3 confirmed the importance of platelets in enhancing the L. E. cell phenomenon. If clotting was allowed to proceed with only the platelets as the variable, the intensity of the L. E. cell phenomenon varied directly with the concentration of platelets.

Of course, clotting of the thrombocytopenic plasmas led to poor yields of thrombin and of activated labile and stable factors. However, the experiments summarized in table 2 had already eliminated both thrombin and the accelerator factors from consideration.

Finally rabbit brain thromboplastin, when substituted for platelets in each of these experiments, gave comparable results. Thus it seems possible that the thromboplastic factor of platelets is the significant fraction.

Whether this factor ever exists free, as such, is not known; it is possible, or even probable, that its liberation from intact platelets is occasioned by its combination with one or more of the plasma thromboplastin factors. It is thus possible that this L. E. cell cofactor contains elements derived from the plasma as well as platelets. Experiments to determine the existence and nature of possible plasma co-factors are under way.

The present studies have shown that there is a co-factor necessary for the
activity of the L. E. cell factor, that this co-factor is, at least in part, derived from platelets, and that it is probably thromboplastin or one of the intermediate products formed during the elaboration of thromboplastin.

This is in accord with the theory advanced by Zimmer and Hargraves and helps to explain several hitherto poorly understood facts about the L. E. cell phenomenon:

(1) It has never been observed in direct smears of blood or bone marrow, even though all of the elements necessary for its production are already present.*

(2) It has been produced in vivo at the sites of artificially induced inflammatory lesions; inflammation is often associated with some fibrin formation.

(3) Hematoxylin bodies (considered by Klemperer et al. to be a manifestation of the L. E. cell phenomenon) are consistently observed to be most frequent in the neighborhood of inflammatory lesions and are always very striking in the endocardial vegetations of the Libman-Sacks type.

(4) Inconsistencies in the L. E. cell phenomenon intensity observed from day to day may possibly be explained on the basis of ease of venepuncture and other details of technic not hitherto considered important.

Kurnick has recently reported the finding of a serum cofactor for the L. E. cell phenomenon. The relationships among these factors, the "plasma thromboplastin" of Campbell and Stefanini and the L. E. cell co-factor derived from platelets, are currently being investigated.

Activation of platelets with release of their thromboplastic factor is the initiating event in the coagulation process. Our findings have shown that at least this initial step in clotting is necessary for the L. E. cell phenomenon to take place. Two cases of systemic lupus with circulating anticoagulants which functioned as antithromboplastins were reported by Conley. The activation of the L. E. cell factor which occurs when plasma is allowed to react with platelets might be a necessary part of the coagulation mechanism. The anticoagulant might then be the L. E. cell factor itself. A systematic survey of blood clotting in our cases of systemic lupus is under way and will be reported in a subsequent publication. Preliminary results tend to confirm this hypothesis.

**SUMMARY**

A factor derived from blood platelets has been shown to be necessary for the activation of the L. E. cell factor. This L. E. cell cofactor may be identical with the platelet thromboplastic factor. The relation of these findings to occurrence of the L. E. cell phenomenon in vivo and to the occurrence of a coagulation defect in systemic L. E. has been discussed.

**SUMMARIO IN INTERLINGUA**

Nos ha demonstrate que un factor relaxate sub certe conditiones per le plachet-tas sanguinee es necessari pro activar le factor del phenomeno del cellula a lupus erythematose. Illo age como co-fact-or in iste phenomeno e es forsan identic con le factor thromboplastic del plachettas. Nos discute le relation de iste cons-

* An exception to this rule has recently been published, a case in which L. E. cells were observed agonally in direct smears of peripheral blood.
REFERENCES
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