A Comparative Evaluation of the Sensitivity of the L.E. Cell Test Performed Simultaneously by Different Methods

By Edmund L. Dubois and Vivian Freeman

Since Hargraves' classic description of the L.E. cell in 1948, numerous methods for the performance of this test have been described by various authors. As each technic appeared its proponents felt that their method was the most sensitive. In view of the conflicting reports and the necessity to determine the comparative merits of the various methods, it was decided to perform simultaneously a battery of different types of L.E. tests on peripheral blood drawn from one venipuncture and to study the relative importance of anticoagulants, preservatives, clotting, and leukocyte trauma on the L.E. phenomenon.

Materials and Methods

During the first phase of this study the following four technics were utilized after obtaining 30 cc. of peripheral blood from a single venipuncture: 10 ml. was added to each of two test tubes containing 0.75 and 3.75 mg. of aqueous sodium heparin, another 10 cc. was permitted to clot, and finally several drops were used to perform the Snapper-Nathan ring technic described below. The amounts of anticoagulant could be approximated by using three drops of aqueous sodium heparin 10 mg. per ml. and 50 mg. per cc. obtained through a 21 gauge needle. The preservative contained in the 50 mg. heparin was phenol 0.5% and in the 10 mg. concentration was chlorobutanol 0.5%. Outlined below is the method by which we performed the test utilizing heparinized blood.

1) The heparinized blood was allowed to remain at room temperature 30-60 min.

2) The specimen was then centrifuged at approximately 1000 RPM or (104 X G) for five minutes, or until a clear separation of the three layers was obtained.

3) The supernatant plasma was removed with a Wintrobe pipet and discarded.

4) One ml. of the buffy coat was taken and placed into a Wintrobe tube.

5) The Wintrobe tube was centrifuged at 1000 RPM or (104 X G) for five minutes, or until a separation of the three layers was obtained.

6) The supernatant plasma was again removed with a Wintrobe pipet and discarded.

7) The buffy coat was carefully aspirated into the pipet and then smeared on glass slides. In order to make a thin section of the smear, the smearing slide was pushed forward and then pulled backward over one-half the width of the blood film. This enabled one to better study the cytology when the buffy coat was very rich in leukocytes.

8) The preparations were stained with Wright's stain in the usual manner.

9) The film was examined especially at the edges for at least 10 minutes under oil immersion lens. In this length of time, several thousand leukocytes would be studied. If any abnormalities were found, the slide was reviewed for a longer period and further preparations made.

The clot technic was performed as follows:

1) 10 ml. of peripheral blood was permitted to clot and to remain at room temperature for two hours.

2) The clot was loosened from the tube if necessary by wooden applicator sticks and

From the Department of Internal Medicine, University of Southern California School of Medicine and the Los Angeles County General Hospital, Los Angeles, California. Supported by a grant from the Attending Staff Association of the Los Angeles County General Hospital.

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then completely macerated through a fine tea sieve, 30 mesh per inch, using a test tube as a pestle. This latter step was most important.

Zimmer and Hargraves stated in their original paper that the clot might also be broken up with wooden applicator sticks. This was the technic which I originally performed and reported in a previous study comparing heparinized and clot methods and the results were opposite to those currently to be presented. Zinkham and Conley also disrupted the clot with applicator sticks in their comparative study of the clot vs. rotary methods and this could account for their finding fewer L.E. cells by clot method. We simultaneously performed the clot technic utilizing these two methods of macerating the clot in specimens from eight different patients. In every case the sieved method contained many more L.E. cells and more leukocytes. The probable reason for this will be discussed later.

3) The sieved material was placed in a standard test tube and centrifuged at 1000 RPM or (104 X G) for five minutes or until a clear separation of the three layers was obtained. From this point on the same technic was employed as in the heparinized methods. We continued with Step 3 as outlined in the previous technic.

Snapper and Nathan have shown that glass slides have an affinity for leukocytes when clotted blood rests on them. Our modification of their technic was performed as follows:

1) Substrate slides of normal leukocytes were prepared by permitting a few drops of blood to clot for one hour within a rubber ring approximately 0.8 cm. in diameter and 0.2 cm. high, placed on a glass slide. Two rings were set on each slide. The slides were placed in a Petri dish, the bottom of which was covered by moistened filter paper. After incubating at room temperature for one hour, the clot and ring were removed by pushing them off and the slide was washed with serum to remove the excess free red cells. The use of saline as a wash would distort the leukocytes. The slides were then dried. They have been kept at room temperature as long as five weeks without losing their potency.

2) In order to test the blood of a suspected patient, a small piece of number 2 coverslip was placed on either side of the substrate area as a pillar. Then a large drop of finger blood was put on a square 22 mm. wide coverslip and inverted over the substrate area resting on the glass pillars. When a battery was drawn, venous blood was used for the preparation.

3) The preparation was incubated at room temperature for 2 hours in a Petri dish, the bottom of which was covered by a piece of moistened filter paper.

4) The coverslip was removed by pushing it off the glass slide. The substrate was washed to remove excess red cells with either the patients serum if it was available or normal serum. This step was not always necessary. Saline should not be used as a wash, as it distorted the cytology and caused poor staining.

5) The slides were stained with Wright's stain in the usual manner.

Interpretation of the L.E. Cell Tests

There are four changes that are of significance in the preparations, namely, increased rouleaux formations, "hematoxylin bodies", rosettes and L.E. cells. These changes have been detailed in numerous publications with good illustrations and only the significant differential points will be reviewed here. Increased rouleaux formation is nonspecific.

"Hematoxylin" bodies are free ovoid masses of dark purplish staining material which may vary in size from a fraction of a single leukocyte to masses of this material which may be the diameter, of a dozen white cells. They are cytochemically identical to the tissue hematoxylin bodies. The finding of these masses in L.E. cell preparations is not pathognomonic. Their appearance in the heparinized and clotted preparations was similar. Many more were usually found by the latter method. The reasons for this will be discussed later. Those seen in the clotted preparation seemed to stain darker and be smaller in size probably because of maceration through the sieve. Free hematoxylin bodies were less frequently seen
in the Snapper-Nathan ring method than by the clot technic and were least common in the heparinized technic. The smallest number were found with the higher heparin concentrations (see table 4).

Rosettes are clusters of polymorphonuclear leukocytes surrounding a hematoxylin body. These were found more frequently in the clotted than in the heparinized methods. Their appearance was identical. Rosettes were very rarely found in the ring method. Often rings of white cells may surround platelet clumps. This is a pseudo-rosette and is not significant. The appearance in a preparation of a typical rosette is highly suggestive, but again, not diagnostic of lupus and further studies are warranted. The smallest number of rosettes were found with the use of a greater amount of heparin (see table 4).

The change that must be present in order to interpret a preparation as positive is the typical L.E. cell. We considered the test positive only if a minimum of two classic examples were found. The cytology of the L.E. cell was best studied in the heparinized preparation, for here they were the most characteristic. When seen in the clotted preparations, the cells were often present in clumps with multiple inclusions being seen far more frequently than in the heparinized technique (see figures 1–4). Many more “tart” cells were found in the clotted method and often atypical “tart” cells which have some diminution in chromatin structure of the inclusion but not a complete absence of it (see figure 5). If we were able to find only a few of these atypical “tart” cells in the preparation and never discovered more typical cells in the remainder of the battery, the test was called negative. The clot method was the most difficult to interpret because of these

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Fig. 1. Typical L.E. cells seen in heparinized preparation (900X).
Fig. 2.—Clusters of L.E. cells with single and multiple inclusion bodies characteristic of the pattern often seen in the sieved clot (1000X).

Fig. 3.—Snapper-Nathan L.E. preparation. Note multiple inclusions in all the cells (600X).
Fig. 4.—L.E. cell with two inclusion bodies, one or both of which may be due to nuclear degeneration without phagocytosis. Sieved clot preparation (2000X).

Fig. 5.—Tart cell (2500X). Note detailed chromatin structure in the inclusion body.
changes and in doubtful cases verification must be obtained with other technics. So called "pre" L.E. cells which are leukocytes with pyknotic nuclei are also not significant. 15

The ring technic, as illustrated in figure 3, produces startling results when positive. The L.E. cells were again slightly different from those seen in other preparations. There were usually many small inclusions with varying degrees of homogeneity of their chromatin network. The leukocytes and their inclusions were also much larger than those seen by the other methods.

Study of the bone marrow by heparinized or clotted methods may still be of value when L.E. cells cannot be demonstrated by these technics. Since utilizing the above battery or the revised battery with the Zinkham-Conley technic discussed below, we have not seen any patients who had positive marrow tests and negative peripheral blood tests.

Results of the First Battery of L.E. Tests

Table 1 summarizes the results of 68 simultaneous batteries performed on 44 proven cases of systemic lupus erythematosus (SLE). Nine patients were untreated at the time of their first series of tests and the other 35 had received some therapy. The most sensitive technique was the sieved clot which was positive in 41 batteries or 60.3 per cent of the group. In ten batteries it was the only positive test. The next most sensitive test was the Snapper-Nathan ring technic which was positive in 42.7 per cent of the entire series, and was the only positive test of the battery in 4 cases. It is obvious that the originally used heparinized methods are the least sensitive, however, in two cases, the 0.75 mg. heparin technic was the only positive test in the group. On other batteries of tests performed earlier or later on these two patients, either the clot or the ring was also positive. The method of employing 3.75 mg. of heparin as anticoagulant was discontinued at this point because of its lack of sensitivity.

Since there was a marked decrease in the numbers of L.E. cells with increasing amounts of heparin, the next step was to quantitate the amounts of anticoagulant which inhibited the L.E. phenomenon. After this study was completed, Haserick in a comparison of L.E. cell technics stated that excessive amounts of heparin inhibited the L.E. phenomenon but he did not state the amounts used. 16

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of Positive Tests</th>
<th>Positive Per Cent</th>
<th>Mean No. L.E. Cells 1,000 WBC</th>
<th>% Positive Only Positive Batteries</th>
<th>No. of Times only Positive Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sieved Clot</td>
<td>41</td>
<td>60.3%</td>
<td>20.0</td>
<td>87.4%</td>
<td>10</td>
</tr>
<tr>
<td>Heparin (0.75 mg)</td>
<td>25</td>
<td>36.8%</td>
<td>2.2</td>
<td>53.1%</td>
<td>2</td>
</tr>
<tr>
<td>Heparin (3.75 mg)</td>
<td>21</td>
<td>30.9%</td>
<td>1.6</td>
<td>44.7%</td>
<td>0</td>
</tr>
<tr>
<td>Ring</td>
<td>29</td>
<td>42.7%</td>
<td>16.4</td>
<td>61.7%</td>
<td>4</td>
</tr>
</tbody>
</table>

All tests were negative in 21 batteries.
All tests were positive in 13 batteries.
* Positive per cent of 47 batteries in which at least one test by any technic was positive.
The Effect of Anticoagulants and Preservatives on the L.E. Phenomenon

The L.E. phenomenon has been inhibited by drastic changes in pH, preservatives such as alcohol, formalin, phenol, etc., and many other agents such as atabrine and other antimalarials. In order to determine the relative merits of double oxalate and heparin as anticoagulants, in addition to the battery described above, 5 ml. of blood was added to tubes containing ammonium oxalate 6 mg., potassium oxalate 4 mg, both with and without formalin 1 mg. The standard parenteral liquid heparin which was used as a control for these studies also contains a preservative, 0.5 per cent chlorobutanol or 0.37 mg. per 0.75 mg. of heparin. Listed in table 2 are the results of this study. It was apparent that oxalate partially inhibited the production of L.E. cells especially in the presence of formalin. The small amount of chlorobutanol used as a preservative for the parenteral heparin did not seem to significantly inhibit L.E. cell formation.

The next problem was to study the effect of varying concentrations of aqueous heparin which already contains 0.5 per cent chlorobutanol as a preservative and the powdered “pure” material on the formation of L.E. cells. Heparin is a crude substance with many contaminants introduced in the process of extracting it. Eight patients had complete L.E. batteries performed and in addition, 5 cc. amounts of their blood were added to varying concentrations of both aqueous and dry heparin from 0.375 mg. to 6.0 mg. as shown in table 3. Utilizing the type of aqueous heparin (chlorobutanol 0.5 per cent as preservative) which we employed the number of L.E. cells markedly decreased whenever the concentration of heparin was increased over 1.0 mg. per 5 cc. of blood. With the dry powdered heparin the amount which inhibited the L.E. cells was 0.75 mg. of heparin or over. There was actual destruction of leukocyte morphology and loss of cytological details with concentrations of over 1.5 mg. of aqueous heparin which progressed with increasing concentrations. Similar changes were noted at an equivalent concentration of powdered heparin. Heparin, therefore, in higher concentrations than necessary to produce an anticoagulant effect was an inhibitor of the L.E. phenomenon. It is important to avoid the common practice of heparinizing a

<table>
<thead>
<tr>
<th>Patient</th>
<th>Oxalate with Formalin 1 mg.a</th>
<th>Oxalate without Formalin</th>
<th>Heparin with Chlorobutanol 0.37 mg.</th>
<th>Dry Heparin without Preservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. M.</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>H. R.</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>F. H.</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>H. C.</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>G. H.</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

a 5 ml. of blood was used in each test. The exact formulae in each bottle was as follows: for the oxalate 6 mg. of ammonium oxalate, 4 mg. of potassium oxalate with or without 1 mg. of formalin; and for the heparin containers 0.375 mg. of powdered heparin or aqueous heparin 0.375 mg. with 0.337 mg. of chlorobutanol.

b The number of L.E. cells per 500 leukocytes were counted for the table.
EVALUATION OF THE L.E. CELL TEST

**Table 3. Aqueous Heparin with Chlorobutanol 0.5% as Preservative**
(Average of values obtained in four patients).

<table>
<thead>
<tr>
<th>Concentration</th>
<th># of L.E. Cells/500 WBC</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.375 mg/5 c.c.*</td>
<td>2.5</td>
<td>Beginning WBC destruction with loss of normal cytology.</td>
</tr>
<tr>
<td>0.75</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>1.25</td>
<td>Increasing WBC destruction.</td>
</tr>
<tr>
<td>6.0</td>
<td>0.75</td>
<td>Further WBC destruction.</td>
</tr>
</tbody>
</table>

**Dry (Heparin (Wilson) without Preservative**
(Average of values obtained in four patients)

<table>
<thead>
<tr>
<th>Concentration</th>
<th># of L.E. Cells/500 WBC</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.375 mg/5 c.c.</td>
<td>2.25</td>
<td>Beginning WBC destruction with loss of normal cytology.</td>
</tr>
<tr>
<td>0.75</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>0</td>
<td>Increasing WBC destruction.</td>
</tr>
<tr>
<td>6.0</td>
<td>0</td>
<td>Further WBC destruction.</td>
</tr>
</tbody>
</table>

* Equivlent to 0.75 mg/10 ml. of blood used as routine heparinized L.E. preparation anticoagulant.

* In only two patients were L.E. cells seen at this concentration.

* Only one patient had L.E. cells at this concentration.

syringe or “adding a few drops of heparin” as an anticoagulant since the amounts used may block the L.E. phenomenon. This is particularly necessary to observe when collecting bone marrow for L.E. studies where only a few ml of marrow may be added to a large amount of anticoagulant. Varying concentrations of anticoagulants may account for the occasionally noted phenomenon that specimens taken from peripheral blood may be positive whereas bone marrow samples negative and vice versa.

**Zinkham-Conley Rotary Method**

While our study of the initial four tests was underway, Zinkham and Conley published their technic of rotating the heparinized blood with 4 mm. diameter glass beads and demonstrated the importance of trauma in increasing the number of L.E. cells. This explains the merits of macerating the blood clot through a fine sieve instead of breaking it up with wooden applicator sticks where trauma is less complete. The following modification of the Zinkham-Conley technic was utilized since larger quantities of blood could be used yielding richer buffy coats.
1) 10 ml. of venous blood was placed in a test tube containing 0.75 mg. of aqueous heparin (3 drops of 10 mg./ml. obtained through a 21 gauge needle) and 10 glass beads 4 mm. in diameter.

2) The specimen was incubated at room temperature for 90 minutes. (See table 3.)

3) The test tube was rotated in a modified Shen type rotator which holds 4 standard test tubes (see fig. 6). The machine utilized by us was built to rotate at 50 RPM.

4) The tube was removed from the rotator and centrifuged at 1000 RPM or (104 X G) for 5 minutes or until there was a clear separation of the three layers. We continued from this point with step three of the first technic described in this paper.

Since we already demonstrated the fact that larger doses of heparin inhibit the L.E. phenomenon, a revised simultaneous battery of L.E. tests was again performed utilizing the following four tests: 0.75 mg. of heparin as an anticoagulant per 10 ml. of blood without rotation as described in the first part of this paper, the Zinkham-Conley modified technic discussed above, the two hour sieved clot and the Snapper-Nathan Ring.

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**Fig. 6.—Rotator used for modified Zinkham-Conley method, 50 RPM**
Results of the Second Battery of L.E. Tests

Table 4 shows the detailed findings in a group of 63 batteries performed on 47 proven cases of SLE, both treated and untreated. In 42 batteries there was at least one positive test. In 5 batteries only the rotary method was positive, in 4 the clotted method and in 2 the ring. The plain heparinized method was never the only positive test and hence it has been discarded for routine use.

Very often when equivocal L.E. cells were seen by the clot technic, their typical appearance in the rotary method confirmed our clinical suspicion and the diagnosis. In determining the numbers of each part of the L.E. phenomena 500 white cells were counted in the most positive section of each smear, usually at the edges.

Table 4.—Revised Comparative Study of L.E. Cell Test Techniques in 47 Proven Cases of SLE

(63 Batteries Performed on Both Treated and Untreated Patients)

<table>
<thead>
<tr>
<th>Technic</th>
<th># of Positive Tests</th>
<th># Positive Tests</th>
<th>Mean # of L.E. Cells 500 WBC</th>
<th>Mean # of H. Bodies 500 WBC</th>
<th>Mean # of Rosettes 500 WBC</th>
<th>% Positive</th>
<th># of Times Only Positive Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sieved Clot</td>
<td>31</td>
<td>49.2</td>
<td>7.4</td>
<td>3.6</td>
<td>0.1</td>
<td>74.0</td>
<td>4</td>
</tr>
<tr>
<td>Heparin (0.75 mg.)</td>
<td>15</td>
<td>23.8</td>
<td>1.1</td>
<td>0.6</td>
<td>0.02</td>
<td>35.8</td>
<td>0</td>
</tr>
<tr>
<td>Ring</td>
<td>26</td>
<td>41.3</td>
<td>9.8</td>
<td>0.8</td>
<td>0.02</td>
<td>62.0</td>
<td>2</td>
</tr>
<tr>
<td>Rotary</td>
<td>34</td>
<td>54.0</td>
<td>10.9</td>
<td>10.5</td>
<td>0.1</td>
<td>81.0</td>
<td>5</td>
</tr>
</tbody>
</table>

* Positive per cent of 42 batteries in which at least one test by any technic was positive.

Fig. 7.—Cluster of L.E. cells and hematoxylin bodies typical of those seen by the Zinkham-Conley method. Note multiple small inclusions (1000X).
As shown in table 4, the incidence of L.E. cells rose from a low of 1.1 per 500 white cells in the plain heparinized specimen to 10.9 per 500 leukocytes after rotation. The sieved clot had 7.4 cells per 500 white cells and the ring 9.8 per 500. The mean number of hematoxylin bodies showed a similar pattern with 0.6 per 500 cells in the heparinized and 10.5 in the rotated specimen. The numbers of rosettes were very few as shown on the table. Typical rosettes were infrequently encountered on positive L.E. smears. Many clumps of leukocytes were seen and clumps of polymorphonuclear leukocytes surrounding platelet masses or pseudo-rosettes. Figure 7 shows the high concentration of L.E. cells with multiple inclusions and hematoxylin bodies often seen in the rotary method. There were 42 batteries in which at least one test was positive. The plain heparinized specimen was never the only positive method in this series. In 17 batteries the plain heparinized specimen was negative while the same blood specimen when rotated was positive. This confirmed Zinkham's and Conley's findings.

In view of these results we now use only the following three tests to screen strongly suspected cases of SLE, the Snapper-Nathan ring, the sieved two hour clot and Zinkham-Conley modified technic. If only one test can be performed the latter method is the most sensitive. In practice at the Los Angeles County General Hospital, the rotary glass bead technic is routinely performed on all requests for L.E. cell preparations. If the case is clinically very suggestive of SLE or if there are suspicious findings on the rotary L.E. preparation then the full battery of peripheral blood L.E. tests is performed. If this is still negative, bone marrow is tested by the modified Zinkham-Conley and clot methods. As stated before no patients have yet been seen in whom the peripheral battery was negative and the bone marrow tests positive. Eight patients with clinical SLE whose peripheral battery was negative have also had a marrow battery. This too was negative.

**Table 5.—Effect of Placement of the Rotating Period upon the L.E. Phenomenon**

(Average Values of 5 Patients)

<table>
<thead>
<tr>
<th></th>
<th>L.E. Cells*</th>
<th>Hematoxylin Bodies*</th>
<th>Rosettes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Immediately after blood is drawn rotate specimen for 30 min. and then make L.E. preparation</td>
<td>7.4</td>
<td>32.4</td>
<td>0.6</td>
</tr>
<tr>
<td>2. Immediately after blood is drawn rotate specimen for 30 min. Let stand 90 min., then make L.E. preparation</td>
<td>22.2</td>
<td>17.6</td>
<td>0.4</td>
</tr>
<tr>
<td>3. 30 min. after blood is drawn, rotate specimen for 30 min. Let stand 60 min., then make preparation</td>
<td>27.4</td>
<td>32.0</td>
<td>1.0</td>
</tr>
<tr>
<td>4. 60 min. after blood is drawn, rotate specimen for 30 min. Let stand 30 min., then make preparation</td>
<td>22.0</td>
<td>34.6</td>
<td>0.0</td>
</tr>
<tr>
<td>5. 90 min. after blood is drawn, rotate specimen for 30 min., centrifuge and make preparation</td>
<td>18.2</td>
<td>24.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Per 500 white blood cells.
EVALUATION OF THE L.E. CELL TEST

The Effect of the Placement of the Rotating Period on the L.E. Phenomenon

Since it has already been well demonstrated that trauma to the white cells increases the L.E. phenomenon, the next problem which arose was when would it be best to rotate the specimen in order to obtain the maximum number of L.E. cells. This was studied in five positive cases as shown in table 5. It was apparent from this data that the specimen should be permitted to incubate at least 30 minutes both before and after rotating for maximum L.E. cell production, prior to centrifugation.

The Effect of Treatment on the L.E. Cell Test

The second type of L.E. battery discussed above consisting of 4 tests was performed on forty patients who had had prior positive L.E. tests. After two months of treatment, eighteen patients had completely negative batteries, seven patients had fewer than 5 L.E. cells per 500 leukocytes on any one test and fifteen had more than that number. Therefore, approximately fifty per cent of the patients have either a complete or almost complete disappearance of their cells after two or more months of therapy.

Summary

1) A group of at least three types of L.E. cell tests should be performed in order to adequately screen strongly suspected cases of SLE. This battery consists of the sieved two hour clot, the Snapper-Nathan ring method and the Zinkham-Conley rotary glass bead technic. Utilizing these three methods in studying a group of 47 proven cases of SLE both treated and untreated, 63 batteries were performed. In 42 of these batteries there was at least one positive test. In 5 batteries only the rotary method was positive, in 4 the clotted method and in 2 the ring method. The largest numbers of L.E. cells and hematoxylin bodies were found by the rotary method. If only one test is to be performed this latter technic is the method of choice.

2) The use of excessive amounts of heparin over 0.75 mg./10 cc. of blood resulted in a depression of the L.E. phenomenon. The technic of adding “small amounts” of heparin to blood or “heparinizing a syringe” to draw specimens for L.E. studies is to be avoided.

3) The explanation for the augmentation of the L.E. phenomenon by clotting or rotation with glass beads seemed to be the production of leukocyte trauma by either method. This explained the poor results obtained when the clotted blood specimen is broken up with wooden applicator sticks rather than macerated through a fine sieve. The clotting mechanism itself probably has little to do with the increased numbers of L.E. cells.

4) With adequate treatment the L.E. cells disappeared in many patients. Forty patients with positive L.E. cell tests treated two months or more had a simultaneous battery performed on them as described above. All the tests were negative in eighteen patients, seven patients had fewer than 5 L.E. cells per 500 leukocytes and fifteen patients had more than that number.

Summario in Interlingua

1. Al minus tres typos de tests pro cellulas de lupus erythematose (L.E.) debereca esser executate pro probar adequatemente un date caso que es forte-
mente suspecte de systemic lupus erythematose. Le batteria del tests in question consiste de (1) le test a cribratio de coagulos de duo horas de etate, (2) le test a anulo de cauchu de Snapper e Nathan, e (3) le test a rotation con perlas de vitro secundo Zinkham e Conley. Iste tres methodos eseva utilisate in le studio de un grupo de 47 provate casos de tractate e non-tractate systemic lupus erythematose. Un total de 63 batterias de tests eseva executate. In 42 batterias, al minus un del tests eseva positive. In 5 batterias solmetut-e he metodo rotatori eseva positive, in 4 le methodo a coagulo, c in 2 le methodo a anulo. Le plus grande numero de cellulas de L.E. e de corpores hematoxylinic eseva trovate per le methodo rotatori. Si on debe restringer se a un sol technica, iste ultime es le technica de election.

2. Le uso de excessive quantitates de heparina (plus que 0,75 mg pro 10 cm³ de sanguine) resultava in le depression del phenomeno de L.E. Le technica del addition de “parve quantitates” de heparina al sanguine o de “heparinisar le syringa” pro le transferimento de specimens de sanguine in studios in re L.E. debe esser evitate.

3. Le explication del intensification del phenomeno de L.E. per coagulation o rotation con perlas de vitro pareva trovar se in le production de traumatisation de leucocytos per ambe iste methodos. Isto explica etiam le inferioritate del resultatos obtenite quando le specimen de sanguine coagulate es disarmite per medio de applicatores de ligno plus tosto que macerate per medio de un cribro. Il es probable que le mechanismo coagulatori per se ha pauc a facer con le augmentate numero del cellulas de L.E.

4. In multe patiemutes, formas adequate de therapia resultava in le disparition del cellulas de L.E. Quaranta patiemutes con positive tests pro cellulas de L.E. eseva tractate durante duo menses o plus. Alora le supra-describe batterias de tests eseva executate pro illes. In 18 patienstes, omne le tests eseva negative. Septe patienstes habeva minus que 5 cellulas de L.E. in 500 leucocytos. December patienstes habeva plus que ille msumero de cclhulas de L.E.

REFERENCES


EVALUATION OF THE L.E. CELL TEST

A Comparative Evaluation of the Sensitivity of the L.E. Cell Test Performed Simultaneously by Different Methods

EDMUND L. DUBOIS and VIVIAN FREEMAN