A Simple Office Procedure for Demonstrating Lupus Erythematosus Cells in Peripheral Blood

By HAZEL B. MATHIS

A SIMPLE METHOD of demonstrating the lupus erythematosus cell in peripheral blood is presented in this preliminary paper. Of 9 patients with the clinical diagnosis of acute disseminated lupus erythematosus referred for hematologic study, "L. E." cells were demonstrated in large numbers in venous blood in every case. In 18 control cases, (2 multiple myeloma, 1 periarteritis nodosa, 1 dermatomyositis, 3 discoid lupus, 2 rheumatoid arthritis, 2 rheumatic heart disease, 1 pernicious anemia, 1 miliary tuberculosis, and 5 undiagnosed) no "L. E." cells were found. "L. E." cells can be demonstrated with ease and rapidity by this technic since it requires only a small sample of the patient's blood, and simple equipment.

METHOD

1. About 5 cc. of venous blood is withdrawn in the usual manner, and after removal of the needle from the syringe, the blood is placed in a sterile centrifuge tube containing three drops of liquid heparin (Liquaemin Sodium, Organon, Inc.; 1 cc. = 10 mg.). After replacing the sterile cotton pledget, the tube is gently shaken to insure adequate mixing.

2. The tube is allowed to stand in an upright position at room temperature until the blood cells have settled to the bottom. This usually requires about thirty minutes.

3. A long stemmed pipet (such as used for filling Wintrobe hematocrit tubes) is then employed to transfer the supernatant plasma portion, as well as the extreme uppermost level of the underlying cells to a second sterile centrifuge tube. This is held at 37.5 C. for forty-five minutes in either an incubator or water bath. When such facilities are unavailable, merely allowing the tube to remain at room temperature for two hours will suffice. The contents remaining in the first tube are discarded.

4. The incubated plasma is next centrifuged at 1500 to 2000 r.p.m. for three minutes, thus concentrating the buffy coat portion in the narrow tip of the tube. Pipet collection of the buffy coat is thus facilitated. Excessive centrifugation should be avoided so as not to pack the sediment too tightly.

5. The supernatant plasma is removed with a Wintrobe pipet and all but approximately 0.5 cc. discarded. This latter volume is retained for subsequent use as a diluent.

6. The sediment is now completely removed with the pipet. One drop is placed on the surface of a glass slide. Slides should be kept in 95 per cent alcohol and wiped dry just before using. A second glass slide is placed on top and gentle pressure maintained until the drop of sediment is compressed into a thin layer of material. The slides are then drawn apart in opposite parallel directions. Several slides are prepared in the same manner. Usually three drops of sediment may be delivered from the pipet, thus producing six films. The interior of the centrifuge tube is then searched for small cartilage-like masses, composed mainly of collections of packed platelets. These masses are picked up with a small wire loop, placed on a slide, and emulsified with a drop of the plasma diluent. A film is then made as previously described.

From the Division of Hematology, Department of Medicine, New York University Post Graduate Medical School and the Fourth Medical (NYU) Division, Bellevue Hospital, New York, N. Y.
Fig. 1.—Platelet mass preparation of peripheral blood of patient with acute disseminated lupus erythematosus. Note large number of "L. E." cells clumped about platelets. (X 460).

Fig. 2.—Same preparation showing details of "L. E." cells. (X 1794).

7. All preparations are air dried and stained. While any of the Romanowsky stains may be used, our best results have been obtained with Tetrachrome (MacNeal) stain. Stain is applied for one and a half minutes and an equal volume of distilled water added for an additional two minutes. Slides are washed under running water and drained dry.
LUPUS ERYTHEMATOSUS CELLS IN PERIPHERAL BLOOD

8. The films are examined first under "high dry" power, "L. E." cells being more readily found at the periphery of the film. Figures 1, 2 and 3 are photomicrographs of typical "L. E." cells.

Fig. 3.—Detail of preparation from another case showing "L. E." cell caught in platelet mass. (X 1794).

Table 1.—Number of "L. E." Cells per 100 High Power Fields

<table>
<thead>
<tr>
<th>Case</th>
<th>Buffy coat films</th>
<th>Platelet mass films</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>22</td>
<td>87</td>
</tr>
<tr>
<td>2.</td>
<td>17</td>
<td>105</td>
</tr>
<tr>
<td>3.</td>
<td>38</td>
<td>49</td>
</tr>
</tbody>
</table>

Remarks on Technic

Difficulty in finding "L. E." cells in buffy coat films of peripheral blood has been mentioned by other workers.1-6 We believe this difficulty is often the result of the cells having been trapped in the platelet masses, (figure 3) and in such instances, they are more readily found in the latter. Table 1 shows a striking disparity in the number of cells found in films of the buffy coat and films of the platelet masses from the same sample of blood.

Heparin is used as the anticoagulant since it is less destructive to leukocytes than any other anticoagulant. Although "L. E." cells have been demonstrated by using a mixture of ammonium and potassium oxalate, and by simply mixing blood with isotonic saline to prevent coagulation, neither has proved as satisfactory as heparin.
The use of two centrifuge tubes of standard type eliminates the necessity for specially constructed equipment. The best results have been obtained when tubes and pipets are sterile so that bacterial clumps in films were avoided. Since absolute dryness is not essential, sterilization by boiling is sufficient.

Experience indicates that incubation should not be too greatly prolonged. In 2 of these cases in which "L. E." cells were found after incubation at 37.5°C for forty-five minutes, it was not possible to demonstrate these cells on the same specimen after twenty-four hours additional incubation. Storage of heparinized blood in a refrigerator for eighteen hours prior to the test has not interfered with the reaction.

The advantages of this method appear to be as follows:
1. It is applicable to the small laboratory or physician's office.
2. No sternal marrow aspiration is necessary.
3. Exclusive use of peripheral blood lessens the chances of encountering unidentifiable cells and is easier to stain due to less fat content.
4. The use of marrow or blood from other sources, such as other patients or laboratory animals, is unnecessary.4-6
5. Results have been consistently reproducible.

**SUMMARY**

A simple but effective method of demonstrating "L. E." cells in the peripheral blood has been described.

**REFERENCES**

Methods: A Simple Office Procedure for Demonstrating Lupus Erythematosus Cells in Peripheral Blood

HAZEL B. MATHIS