The Mechanics of the "L.E." Cell Phenomenon, Studied with a Simplified Test

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THE DISCOVERY of the "L.E." cell in heparinized bone marrow preparations of patients with disseminated lupus erythematosus by Hargraves et al.1 has been repeatedly confirmed2-4. It is the general consensus that this phenomenon appears to be highly specific for this disease5,6.

The "L.E." cell consists characteristically of a neutrophilic polymorphonuclear leukocyte whose nucleus is compressed to the periphery of the cell by the cytoplasmic inclusion of a relatively homogeneous, swollen mass which takes a pale blue color with Wright or Giemsa stains.

Since the original observation of Hargraves, technics have been broadened to demonstrate this phenomenon by various methods. All the current methods involve the necessity for venipuncture, repeated and bothersome centrifuging and the talents of a trained technician.

The preparation of the smears in which the "L.E." cells must be searched for requires hours of preparation. In addition, a time-consuming and careful search of the specimen is commonly necessary before significant numbers of "L.E." cells are found. In some instances, when the diagnosis of disseminated lupus erythematosus is certain on clinical grounds, one may spend several fruitless hours searching for the "L.E." cell.

For these reasons an extremely simple, inexpensive, and rewarding method using one drop of finger blood has been devised and has been described elsewhere8. This technic is not only simple and quick, but it permits the concentration of a large number of "L.E." cells in a small area which facilitates the search for these cells.

Experience with this method brings to light a new concept of lupus cell formation, and may serve to answer some of the perplexing features of this phenomenon.

HISTORICAL

It has gradually become apparent that "L.E." cells are formed when "normal" leukocytes are brought into contact with serum of a patient with lupus erythematosus. For this purpose most investigators have prepared buffy coats of normal blood. However, a much simpler method for the collection of large amounts of white cells exists.

Since 1916, one of us (I.S.), in studying phagocytic activity of normal leukocytes, has observed that large numbers of neutrophilic leukocytes and an occasional eosinophil, basophil, and monocyte could be "concentrated" from one drop of peripheral blood by virtue of the ameboid movements invested in these cells. This was accomplished by placing a few drops of peripheral blood in a shallow rubber ring on a clean glass slide, and incubating this preparation in a

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Submitted January 7, 1955; accepted for publication February 19, 1955.

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moist chamber. After thirty to sixty minutes of incubation, the ring is removed. The clot adherent to the slide is gently washed off with saline or with serum and then quickly dried. After staining with Wright or Giemsa stain the area on which the clot has rested presents a dense aggregation of neutrophilic leukocytes, occa-

Fig. 1.—Saline washed “ring” revealing polymorphonuclear leukocytes and an occasional monocyte. Some of the cells display marked pseudopod formation.
sional eosinophils, and monocytes. The cells can be seen in various stages of ameboid movement and obviously have crept out of the clot (fig. 1).

If a small amount of highly pulverized starch granules is added to the blood before it is placed in the rubber ring, marked phagocytosis of this material by the leukocytes can be observed and the leukocytes which assemble on the slide are filled with starch granules. It is of some importance to note that the nucleus of the phagocyte filled with starch is compressed against the wall of the cell, presenting a picture which is highly reminiscent of the flatly compressed nucleus of the “L.E.” cell (fig. 2).

Technically more satisfactory accumulations of leukocytes can be obtained by making hanging drop preparations. This may be accomplished by affixing a drop of fresh blood on a long cover glass and immediately bringing the drop into contact with the glass slide by supporting the ends of the long cover glass on small pieces of No. 2 cover glasses. This preparation is incubated in a similar manner. When the cover glass is gently removed, the clot which is formed will be either removed with the cover glass or may be carefully peeled away from the slide if it is not initially removed. The stained area will reveal innumerable ameboid white cells free from red cell contamination.

When Hargraves first described the “L.E.” cell, we made efforts to reproduce this phenomenon by allowing lupus blood to clot within a rubber ring as above, placed upon a clean slide and later by making hanging drop preparations of lupus blood. Repeated experiments consistently showed that only normal leukocytes in various stages of ameboid movement crept out of the clotted lupus blood to the surface of the clean slides. No “L.E.” cells were seen among them. It was reasoned that the “L.E.” cells, if formed, were divested of their ameboid properties and remained in the clot. This impression appeared to be confirmed when a rare “L.E.” cell could be found in a smear made from imprints of the clot after its removal. The yield, however, was neither consistent nor striking.

After many trials and errors, different modifications of this technic have been successfully employed for the production of large numbers of lupus cells. The first successful method was based upon considerations which ultimately were proven to be erroneous.

Moyer and Fisher have reported that the number of “L.E.” cells formed during incubation of viable polymorphonuclear leukocytes with lupus serum, is considerably increased when lymphocytes from a patient with lymphatic leukemia are added to the mixture of granulocytes and lupus serum. The increase in the number of “L.E.” cells is greatest when the added lymphocytes are “altered” by previous freezing and thawing. Lee and his co-workers mention the striking augmentation of the “L.E.” cell phenomenon by the subsequent addition of lymphocytes from a case of lymphatic leukemia to a mixture of myeloid cells (from a patient with chronic myelogenous leukemia) and “L.E.” plasma.

We, therefore, attempted to activate the formation of “L.E.” cells from one drop of lupus blood by the addition of leukemic blood. Hanging drop preparations of lupus blood were allowed to come into contact with dried, unfixed, and unstained smears of blood taken from patients with “high count” lymphatic or myelogenous leukemia. Very large numbers of “L.E.” cells were found by this
method. When ordinary smears of normal blood were used as a substrate only a rare, or more often, no “L.E.” cells were found. It thus seemed possible that leukemia blood was in some way essential for the striking results. Further investigation proved that the potent “L.E.” stimulating prowess of leukemic blood
resided only in the large number of white cells with which the hanging drop of lupus blood had been in contact. When aggregations of normal leukocytes were used instead of smears of leukemic blood, the same intense “L.E.” cell formation was obtained.

Since it was necessary for the hanging drop of suspect blood to clot in order to be removed after incubation, white cells obtained from a buffy coat of heparinized or oxalated blood could not be used. It appeared simpler to employ the ring method described above as a source of white cells. The results were unequivocal; inordinately large numbers of “L.E.” cells were found when a hanging drop of lupus blood touched an accumulation of dried, normal leukocytes.

MATERIALS AND METHODS

Twenty-one consecutive cases with the clinical picture of acute disseminated lupus erythematosus in whom “L.E.” cells had been found by the method of Lee⁴ were used for this study.

Thirty healthy persons and fifty patients with various diseases (including various febrile illnesses, blood dyscrasias and the gamut of “collagen” diseases) were selected as control subjects.

For completeness sake we repeat the technic described elsewhere⁸.

Preparation of Substrates

1. Substrates of normal polymorphonuclear cells were prepared by placing a rubber ring with an approximate internal open diameter of 0.5 cm. and height of 0.2 cm. on the center of a clean glass slide. These rings can be simply prepared by cutting them from a rubber tube as used for Bunsen burners. We have commonly cut them from the rubber caps used to stopper the B-D Vacutainer® specimen tubes. The area of the slide enclosed by the ring is filled with one to two drops of finger blood or freshly drawn venous blood from a non-lupus patient. The slide is then gently placed in a petri dish, the bottom of which has been covered by a water-moistened filter paper. The petri dish is covered and kept in a warm spot in the laboratory or, better, incubated at 37°C. The moisture prevents the drying of the clot. After one hour, the slide is removed from the petri dish, held perpendicular to the table top, gently sliding the rubber ring away. The slide is then allowed to dry in this position. The area which had been encircled by the ring contains large numbers of white cells which have crept out of the clot with a variable coating of red cells. These preparations may be used as such but can also be washed with saline or serum. Many such rings can be prepared for future use whenever venous blood has to be drawn for other determinations.

2. Relatively thick smears made from finger blood of various patients with chronic lymphatic, chronic myelogenous, and acute leukemia were also used as substrates. The peripheral total white blood counts varied from 25,000 to over 200,000/cu. mm. These were allowed to air-dry and were not fixed or stained. However, as will be explained presently, the technical results are somewhat better when “rings” of white cells are used as substrate material.

3. Finally, substrate slides were prepared as dried imprints of various carcinomas and also of Hodgkin’s tissue.

Procedure of Test

1. A cover glass, 22 mm. square and of No. 2 thickness, is gently broken into approximate halves between the fingers. Each half is placed approximately 3 cm. apart on the substrate slide. The latter contains either an aggregation of normal leukocytes prepared by the rubber ring method or a leukemia smear. One drop of finger or ear blood from the suspected lupus patient is affixed to the center of a long clean cover glass (24 X 30 mm.) by gently touching the glass to the drop of blood. The hanging drop of lupus blood is allowed to come into contact with the ring of normal white cells or with the leukemia smear by resting the ends of the long cover glass on the broken pieces of cover glass. No. 1 thickness cover
A large number of lupus cells formed from a dried, unwashed "ring" substrate of normal blood.

Glasses should not be used because these do not afford sufficient elevation, and the blood will spread over the slide rather than retain the shape of a drop. The preparation is placed into a moist petri dish chamber as above. After incubation for one or two hours, the slide is taken out of the petri dish and the cover slip gently lifted from its supports.

If leukemia slides or imprints of cancer cells are used as substrate material, the clot fre-
Fig. 4.—Higher magnification of the same preparation showing “rosettes,” “droplet cells” with multiple inclusions, and various stages of lupus cell formation.

sequently will remain affixed to the bottom slide. In the case of ring substrates, consisting of normal leukocytes, the clot will usually be removed with the cover slip. When the clot remains on the substrate slide, it should be removed with a sharp splinter of cover glass. This is best accomplished by holding the slide at a 90° angle with the table and peeling the clot away in toto from above downward. Care should be exerted not to rupture the clot as this
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will contaminate the preparation with too many red cells. After the clot has been removed, the excess serum should be quickly removed by forcefully tapping the slide on the table top. The preparation is quickly dried by forceful waving back and forth. It is then stained by the Wright or after fixation in methanol with the Giemsa methods. The area beneath the clot is examined for “L.E.” cells.

Both “ring” substrates and smears of leukemic blood proved to be effective even when used several weeks after being prepared. This is desirable as this material can be prepared beforehand at one’s leisure and be available when “L.E.” cell formation in the blood of a suspected patient must be tested. It has been our impression, however, that preparations not more than one week old give the best yield of “L.E.” cells.

2. By the addition of a simple maneuver, this method can also be employed to test the formation of “L.E.” cells by serum of a lupus patient. Two drops of normal fresh blood are mixed with an equal amount of suspect serum placed in a small Widal tube. After coagulation, the clot is decanted onto a clean filter paper to remove the excess serum. Two pieces of a No. 2 cover slip are placed on the substrate slide. With a needle, the clot is then positioned on the substrate slide, covered with a cover slip and incubated as before in a petri dish. When this clot is removed and the excess serum and blood cells forcefully removed by tapping, a thin peripheral rim of white cells will be seen to have crept out of the clot onto the substrate slide. This area should be examined for “L.E.” cells.

RESULTS

Control Patients

In none of the normal persons nor in the control disease group was any semblance of the lupus phenomena observed by any of the methods described here. It remains to be seen if, in the future, examples of “L.E.” cell formation by blood or serum of patients not suffering from lupus erythematosus will be encountered. To date we have not had the opportunity to study any patients who have taken Apresoline® for long periods of time.

Lupus Patients

In 20 of the 21 cases with lupus erythematosus, “L.E.” cells, “globs,” and “rosettes” were observed in varying numbers by the Lee method. In all of these cases the clinical picture was classically that of acute disseminated lupus erythematosus. In the remaining patient, in whom the diagnosis remains somewhat in doubt, only a solitary “L.E.” cell was found with the clot method devised by Lee. However, no “L.E.” cells could be visualized after several attempts with the new one-drop method.

When the 20 “good ‘L.E.’ formers” were studied by this method, we found in many cases larger aggregations of “L.E.” cells than either of us had ever observed. In several cases as many as over 100 “L.E.” cells could be seen in a low power field (figs. 3 and 4). These striking results were obtained in all cases using substrates of normal white cells obtained by the “ring” method and with smears of “high count” leukemias. The yield fell off precipitously when the white count of the leukemic substrate source was below 100,000/cu. mm. Neither the maturity nor the type of white cell in the substrate appeared to be of significance providing a rich source of leukocytes was provided. Normal leukocytes, lymphocytes, blasts, and the gamut of myelocytes appeared to be equally efficacious. The predominant lupus phenomenon observed was the formation of the intact “L.E.” cell. However, “globs” and “rosettes,” frequently in very large numbers, were
also seen. In some cases, especially when blood smears of lymphatic leukemia were used as the substrate, “tart” cells were also demonstrable. The presence of these cells was always overshadowed by the formation of large numbers of “L.E.” cells.

A single lupus patient to date has been studied with substrates prepared from various carcinomas and Hodgkin’s tissue. Formation of cells, similar in many respects to lupus cells, was noted in each preparation.

In our hands, normal leukocytes accumulated by the “ring method” proved to be the most satisfactory substrate material. This resided in two technical features inherent in this method: (a) Since the clots usually remained adherent to the cover glass, it was not necessary to perform the dextrous act of peeling the clot away from the substrate slide, and (b) the area of the substrate slide on which the hanging drop had rested was cleared of red cell debris and appeared as a circular window containing lupus cells relatively free from other cellular contamination. This clearing effect is not specific for lupus blood. When a hanging drop of normal blood was positioned upon such an aggregation of normal leukocytes, no “L.E.” cells were formed but the same clearing effect was observed.

Sera from 9 of the 21 lupus patients were studied and gave positive results in 7 instances. The preparations, although containing large numbers of “L.E.” cells, lacked the esthetic appearance of the hanging drop method since there was considerable admixing of red cells with the “L.E.” cells. Serum from the patient who failed to form “L.E.” cells by the hanging drop method also gave negative results with this modification.

DISCUSSION

The method described here appears to be highly effective to isolate, in a relatively simple manner, large numbers of “L.E.” cells from a single drop of finger blood of a lupus patient. It can be mastered by any technician acquainted with routine hematologic technics, in less than an afternoon. It can become a part of routine office procedure, and done with the same finger puncture performed for a routine blood count. Aside from its ease of performance and avoidance of venipuncture, and centrifugation, this method has the advantage of demonstrating large numbers of lupus cells confined to a very small area which considerably facilitates the search.

In addition to the high yield of lupus cells from one drop of finger blood and the ease of performance of this test, we were impressed with the nature of the included mass seen in our preparations. There always are “L.E.” cells present in these preparations which possess all the characteristics of the Hargraves cell. However, in other cells, the inclusion, instead of taking the basic stains, is frequently faintly to strongly eosinophilic. This affinity for acid stains is retained, even if buffers in the alkaline range (pH 7.4) are used with the Wright or Giemsa stains. In addition to its tinctorial dissimilarity, the inclusion in these cells often lacks the “finely-brushed,” homogeneous appearance of the Hargraves cell. Instead, it often appears to be somewhat foamy in character. On the other hand, it is totally divested of any nuclear structure or appearance. Multiple included masses (“droplet cells”) are frequently seen. Its eosinophilic character may or may not suggest a different degree of depolymerization of desoxyribose nucleic
acid than occurs in the inclusion of the other previously described methods. The
inclusions formed by the method described here are Feulgen positive and have
little or no affinity for methyl green stain. The result of these staining methods
seems to indicate that the inclusions of the “L.E.” cells obtained with the method
described here just as the inclusions of the classic Hargraves cells, represent
masses of markedly depolymerized deoxyribose nucleic acid. Detailed photo-
metric studies are in progress to relate the nature of this inclusion body to that
of the classical Hargraves cell.

The character of the cells which are formed when one drop of lupus blood is
brought into contact with a dried imprint of cancer cells requires special dis-
cussion. These cells also consist of polynuclear leukocytes with a nucleus com-
pressed against the periphery of the cell by a large, slightly eosinophilic mass.
However, this mass is much less homogeneous than the inclusion of the typical
Hargraves cell. In Giemsa stained smears, and better still, in Feulgen stained
preparations, the impression is obtained that the inclusion contains several
nucleoli which were originally present in the malignant cell. This observation,
if successfully confirmed, suggests that the depolymerizing effect of lupus serum
is primarily upon the deoxyribose nucleic acid sparing the ribose nucleic acid of
the nucleoli.

One question requires further discussion. Why are no lupus cells formed when
a hanging drop of lupus blood is positioned upon a clean slide, in contrast to the
extensive formation of “L.E.” cells which results when the same hanging drop
is allowed to rest upon an aggregation of non-viable, normal leukocytes? The
following explanation may deserve further consideration.

It is well known that whereas many substances can enter the living cytoplasm,
the living nucleus is impermeable to dyes and other foreign materials. The lupus
factor apparently does not kill white cells, because the leukocytes, which move
out of the clot of lupus blood to the clean slide, are very much alive, and present
vigorous ameboid movements, although they are bathed in lupus serum. Since
these leukocytes are alive, the active factor of the lupus serum apparently cannot
reach the nuclei of these living cells and no depolymerization of deoxyribose
nucleic acid can take place. However, when a drop of lupus blood is positioned
upon a dried “ring” preparation of normal leukocytes or upon a dried leukemia
smear, then the activity of the lupus serum allows for the depolymerization of
the deoxyribose nucleic acid of the nuclei of these dead leukocytes. The latter
material will then be phagocytized by the living polynuclear cells, which creep
out of the hanging drop of lupus blood, and “L.E.” cells are formed.

Since we have found that dried cancer cells and Hodgkin’s tissue imprints can
also be used as substrates, it appears that the deoxyribose nucleic acid of the
nuclei of many different cells can be depolymerized by the lupus factor and be-
come the material from which lupus cell inclusions are formed.

In classic preparations made from bone marrow or blood clots, “L.E.” cells
would then result from the depolymerizing influence of the lupus serum upon the
few leukocytes which become non-viable (or perhaps, immobile) as a result of
the in vitro conditions. The depolymerized nuclei are then phagocytized by the
living leukocytes forming the typical “L.E.” cell. The increase in number of
“L.E.” cells observed when clot preparations are incubated for many hours can
be explained by the gradually increasing numbers of dead leukocytes available for depolymerization. This concept is supported by Moyer's observation\(^9\) that lymphocytes "altered" by freezing and thawing and, also, buffy coats of outdated bank blood containing non-viable lymphocytes, considerably augmented "L.E." cell formation when added to a mixture of freshly prepared neutrophil suspensions and lupus serum.

It is well known that the formation of "L.E." cells is not observed under in vivo conditions; a period of time outside of the body appears to be necessary. "L.E." cells have been found in pleural and peritoneal exudates of patients with disseminated lupus erythematosus. Since only viable leukocytes circulate in the blood it is understandable why "L.E." cells have never been observed in direct blood smears. In exudates, which contain non-viable as well as viable leukocytes, conditions exist which are comparable to the in vitro experiments. In this way the formation of "L.E." cells in exudates can be explained.

Hargraves\(^{11}\) has observed the formation of "L.E." cells and nuclear alteration of cells in the superficial layers of bone marrow and splenic biopsy specimens, respectively. He has reasoned that the lupus factor bathing the peripheral cells is activated by the simulated in vitro conditions in this zone. It also seems possible that the partial drying of the peripheral and superficial layers of bone marrow and splenic biopsy tissue would result in the presence of a few dead hematic cells in these areas. This could be the reason why "L.E." cells were formed in this location only.

We have also considered the possibility that an activating substance is liberated from the dead cells present on the slides. This concept appears less tenable in view of the observation of Kurnick et al.\(^{12}\) that extracts of lysed white cells inhibit lupus cell formation. Further investigation is needed to clarify this problem.

**Summary**

1. A simple method is described for the formation of large numbers of lupus cells by positioning one drop of finger or ear blood of a lupus patient upon an accumulation of dried, normal polynuclear leukocytes. Thick leukemic smears and perhaps even imprints of various carcinomas or Hodgkin’s tissue can also be used as substrates.

2. No false positive results were obtained in a group of control patients.

3. Striking results were obtained with this method in 20 of 21 known cases of disseminated lupus erythematosus. The remaining case in whom a single "L.E." cell was found by a conventional method, to date has yielded no lupus cells by our method.

4. The difference in appearance of the inclusion body of some of these cells from the characteristic Hargraves cell is highlighted.

5. Theoretic considerations of the mechanism of lupus cell formation are discussed and reassessed in the light of our experience with this method. For the formation of "L.E." cells it seems necessary that living polynuclear leukocytes, dead cells and lupus serum be brought together. The lupus serum can depolymerize the desoxyribose nucleic acid of the nuclei of the dead cells, but not of living cells. The depolymerized material is then phagocytized by living polynuclear cells and formation of lupus cells results.
SUMMARIO IN INTERLINGUA

1. Es describite un simple methodo pro le formation de grande numeros de cellulas de lupus erythematose per placiar un gutta de sanguine ab le digito o le aure del patiemte de lupus super un accumulation de desiccate normal leucocytos polymuclear. Spisso frotis leucemic e forsan mesmo impressiones de varie carcinomas o textito de Hodgkini es etiam usabile como substratos.

2. In le grupo de patientes de controlo nulle resultatos pseudo-positive esseva obtenite.

3. Frappante resultatos esseva obtenite con iste methodo in 20 inter 21 casos de previemente confirmate casos de lupus erythematose. In le ultime caso de iste serie un sol cellula de lupus erythematose esseva discoperite per methodos conventional. Usque nunc nostre nove methodo non ha producite ulle cellula de lupus in iste caso.

4. Es signalate le differentia visual del corpore incluse de alicunes de iste cellulas ab illo del caracteristic cellulas de Hargraves.

5. Considerationes teoric del mechanismo del formation de cellulas de lupo es discutite e re-evalutate in le lumine de nostre experientia con iste methodo. A fin que cellulas de lupus erythematose sia formate, il pare necessari che vive leucocytos polymuclear, morte cellulas, e sero lupose es combine. Le sero lupose es capace a dispolymerisar le acido nucleic de disoxyribosa del nucleos de cellulas morte sed non de cellulas vive. Le materia dispolymerisate es tunc phagocytisate per vive cellulas polynuclear con le resultato del formation de cellulas de lupus.

REFERENCES

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