FORMATION OF LARGE NUMBERS OF "LUPUS CELLS" FROM ONE DROP OF PERIPHERAL BLOOD*

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Since the original discovery of the "L. E." cell by Hargraves (1), many different technics for the demonstration of this phenomenon have been published. All these methods are time consuming. Hours of fruitless labor are often spent searching for "L. E." cells in patients in whom on clinical grounds the diagnosis of lupus erythematosus appears certain. It, therefore, seemed justified to devise a new method which permits the concentration of a large number of "L. E." cells in a small area from one drop of finger blood. Due to this concentration of the "L. E." cells, the presence or absence of these cells can be decided after a search of only a few minutes.

MATERIALS AND METHOD

The basis for the method described here depends upon the use of substrate material consisting of aggregations of non-viable white blood cells which may be prepared from leukemic or, even better, from normal blood.

Preparation of substrates

1. Relatively thick smears were made from finger blood of various patients with chronic lymphatic, chronic myelogenous and acute leukemia. The peripheral total white blood counts varied from 23,000 to over 200,000/mm^3. The smears were allowed to air dry and were not fixed or stained. The use of leukemia blood as our initial substrates were based upon the observation of Moyer and Fisher (2) that lymphocytes from a case of lymphatic leukemia acted as a high potency substrate for the formation of "L. E." cells. In subsequent studies with our method we discovered that the high yield of "L. E." cells from leukemia substrates resided in the large numbers of white cells present; normal white cells, if present in quantity, also gave striking results.

2. Concentrated aggregations of normal white cells on a slide 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 cut 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® tubes. The area of the slide enclosed by the ring is filled with 1 to 2 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 filtered paper. The petri dish is covered and incubated at 37° C. The moisture prevents the drying of the clot. After one hour the slide is removed from the petri dish, held at 90° to the table top, gently sliding the rubber ring away. The

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preparation 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 must be used as such without washing with saline or serum. Many such "rings" can be prepared for future use whenever venous blood has to be drawn for other determinations. These "rings" are usually suitable for use up to ten days after preparation.

Procedure of test

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 consists either of a leukemia smear or an aggregation of normal leukocytes prepared by the rubber ring method. One drop of finger or ear blood from a suspected lupus patient is affixed to the center of a long clean cover glass (24 x 50 mm.) by gently touching the glass to the drop of blood. The long cover glass is quickly inverted and the hanging drop is allowed to come into contact with the leukemia smear or with the "ring" of normal white cells by resting the ends of the slide on the broken pieces of cover glass.* It is essential that No. 2 thickness cover glasses should be used because No. 1 cover glasses do not afford sufficient elevation and the drop 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 and incubated for an hour. The slide is then taken out of the petri dish and the cover slip gently lifted from its supports.

If leukemia slides are used as substrate material, the clot usually 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. Should the clot remain on the substrate slide, then it should be removed with a sharp splinter of cover glass. This is best accomplished by holding the slide at a 90° angle to the table and peeling the clot away *in toto* from above downward. Care should be exerted not to rupture the clot as this 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 then dried by forcefully waving back and forth and stained by either the Wright or Giemsa methods. The area beneath the clot is examined for "L. E." cells.

RESULTS

Nineteen "good L. E. formers" were studied by this method and in each case large aggregations of "L. E." cells were found. In several cases as many as 100 "L. E." cells could be seen in a low power field. In one case, in whom the diagnosis of lupus erythematosus remains in doubt, no "L. E." cells were found with this new one drop technic. This patient, however, was examined during a period when the "L. E." cell formation was scanty; notwithstanding repeated trials only one typical "L. E." cell could be demonstrated with the Lee method (3).

* If desired, the cover glass supports and the long cover glass can be held in position by placing a minute dab of vaseline on either side of the cover glass supports.

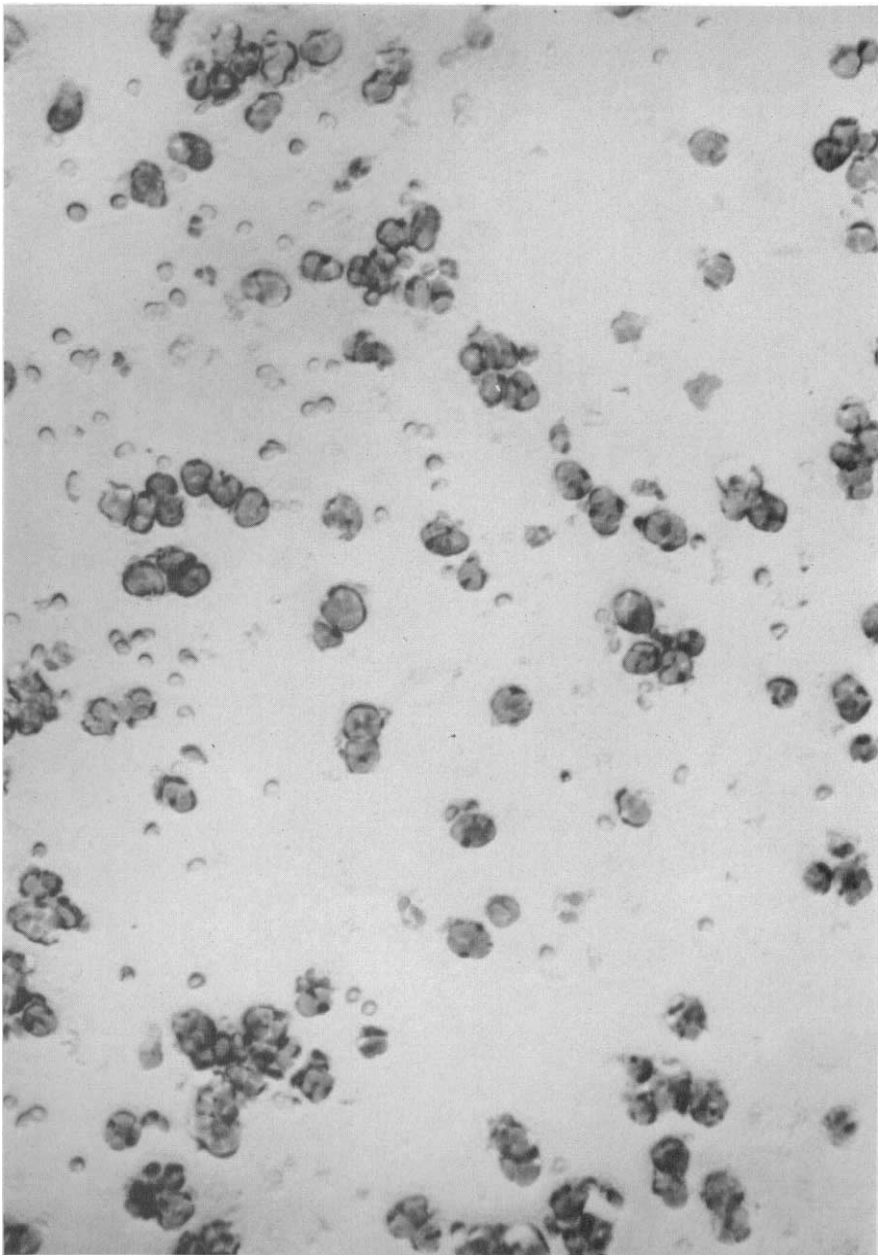

FIG. 1. Lupus cells formed from one drop of peripheral blood, positioned upon ring of normal leukocytes.

On the other hand, neither "L. E." cells nor "tart" cells were found with the new method in a considerable number of normal controls and patients not suffering from lupus erythematosus.

In our hands normal leukocytes accumulated by the "ring method" proved to

be more satisfactory substrate material than the leukemia smears. Firstly, the clots usually remained adherent to the cover glass and the somewhat hazardous peeling of the clot away from the substrate slide was not necessary. In addition, 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 "L. E." cells, relatively free from other cellular contamination.

Many of these "L. E." cells presented all the characteristics of the Hargraves cell. In many other cells the inclusion, instead of taking the basic stains, was faintly to strongly eosinophilic. Multiple inclusions are frequently seen. The inclusion in these cells often lacked the finely-brushed, homogeneous appearance of the Hargraves cell and instead appeared to be somewhat foamy in character.

In addition to the typical Hargraves cells, "tart" cell-like elements were frequently found.

SUMMARY

1. A simple method is described for the formation of large numbers of lupus cells from a single drop of finger or ear blood of a lupus patient.

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

3. Striking results were obtained with this method in 19 of 20 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 in many of these cells from the characteristic Hargraves cell is highlighted.

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