Frozen Cells as Nuclear Source in the L. E. Cell Phenomenon. (23695)

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The L.E. (lupus erythematosus) cell phenomenon(5) consists of two events: the peculiar degeneration of a cell nucleus, and the engulfment of the latter by a phagocyte. The phenomenon apparently occurs if (a) an abnormal factor, present in the gamma globulin fraction(2) of patients with systemic lupus erythematosus, is in contact with (b) cells which in some fashion have been injured, and (c) if active phagocytic cells are available to engulf the transformed nucleus of the injured cell. For reasons not yet apparent, the degenerated nucleus at times is not chemotactic and remains unphagocytosed; when unphagocytosed, this mass is referred to as L. E. material or an L. E. body. A method for producing a large mass of this unique material would allow further study of its chemotaxis (or absence of it), chemical nature, physical properties, and immunologic nature. Direct observation and histochemical analysis of fixed thin blood films have been the major means by which information regarding this unusual cellular phenomenon has been accumulated. The following method, which involves cold injury,* allows the production of large numbers of living L. E. cells and a relatively large quantity of L. E. material suspended in a liquid medium.

Materials and methods. Leukocytes from the circulating blood of human or other species served both as the donors for the altered nuclei and as the phagocytic cells. The leukocytes were separated from 5 ml of whole heparinized blood by the addition of 1.0 ml of 5% dextran† in normal saline solution. Rouleau formation allowed the erythrocytes to fall rapidly to the bottom of the tube, and the supernatant dextran and plasma, containing most of the leukocytes, was removed. Injury to the plasma-suspended leukocytes was then produced by freezing to minus 50°C; it was possible to keep the preparation at this temperature for many months. If the plasma and leukocytes were taken directly from a patient with lupus erythematosus, they were frozen immediately. At times the source of the leukocytes and plasma was some normal human donor, or perhaps another species (*e.g.*, guinea pig), in which case serum from a lupus patient was incubated (37°C) in contact with the cells for one hour prior to freezing. Ordinarily 0.2 ml of serum from a patient with active L.E. was an adequate volume to produce the L.E. cell phenomenon when added to 1.0 ml of normal leukocyte-containing plasma. To allow the final phase of the L.E. cell phenomenon to occur, the frozen material was brought to 37°C, and fresh, active, human leukocytes were added. These were obtained from human blood by the above described dextran technic; gentle centrifugation of the leukocyte-containing plasma fraction (1200 rpm for 10 minutes) followed by decantation of most of the plasma allowed addition of many leukocytes in a small volume. These leukocytes acted phagocytically upon the nuclei which had been injured (frozen) in the presence of the L.E. factor. For microscopic examination, 1.0 ml of the preparation was gently centrifuged in a Wintrobe tube, the sediment then examined. A combination of phase contrast microscopy of an unstained wet preparation and routine Giemsa stain of fixed blood film was the usual means of observation.

Results. By this technic surprisingly great numbers of L.E. cells and free L.E. bodies are produced. If a potent L.E. factor is present, practically every frozen nucleus becomes homogeneous, purple-staining (Giemsa), Fuelgen-positive material which exhibits varying degrees of positive chemotaxis toward freshly added polymorphonuclear and mononuclear leukocytes. The stained L.E. cells (Fig. 1) resulting are in no way different from those

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† Dextraven—Brand of of dextran, produced by Benger Laboratories Limited, Holmes Chapel, Cheshire, England.

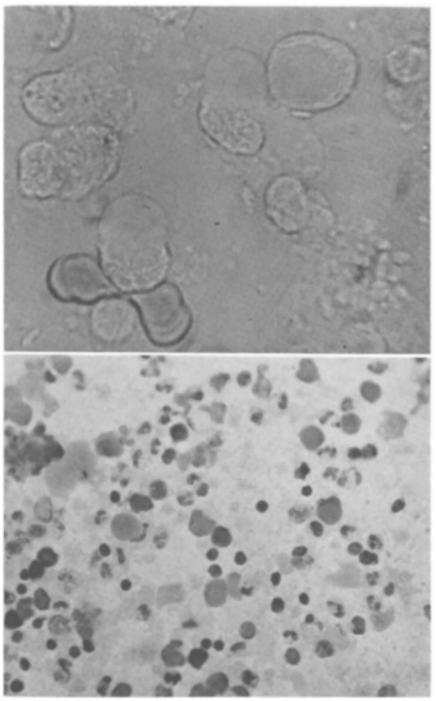

FIG. 1 (top). Two L.E. cells and several free L.E. bodies. Guinea pig cells frozen with L.E. serum. Fresh human WBC added. H & E, × 470.

FIG. 2 (bottom). L.E. cell and L.E. bodies exhibiting chemotaxis. Erythrocytes for size comparison. Phase contrast, × 800.

originally described by Hargraves(1) and produced in various ways by numerous authors in recent years. They exhibit the peripherally compressed, usually multi-lobate nucleus and the engulfed homogenous Fuelgen-positive mass.

Under phase contrast microscopy, observation of the chemotaxis and phagocytosis in the unstained wet preparation provides far more knowledge as to the dynamics of this phenomenon(3,6). Time-lapse micro-cinematography has proved of value in recording and clarifying these events. The L.E. bodies are observed to be globular masses of varying degrees of symmetry, often quite spherical (Fig. 2, 2a). Their margins are distinct, in contrast to the shadowy, often amorphous character of nuclei which have undergone freezing in the absence of the L.E. factor. At times small circular outlines suggestive of nucleolar remnants are seen inside the L.E. bodies. L.E. bodies tend to be free of cytoplasmic remnants. These bodies usually attract phagocytes, sometimes from considerable distances, and at times more than one active leukocyte may be attracted. If several of these cells attach themselves to the L.E. material the size of the resulting mass is quite large. It is in the wet preparations that the diameter of the L.E. cell is most easily observed to be greater than normally circulating formed elements of the blood (Fig. 2).

Unexplained is the fact that some material, seemingly identical in appearance to chemotactic, Fuelgen-positive L.E. material is completely unattractive to active leukocytes in its neighborhood. In some preparations, production of L.E. cells seems to cease only when all available phagocytic cells have transformed themselves into L.E. cells. In rare preparations all of the freshly added leukocytes seem depressed, tending to become rounded, non-motile, and non-phagocytic.

Since the L.E. cell has been defined in respect to its fixed and stained characteristics (5), the living unstained preparation alone without a stained film cannot be utilized diagnostically at this time. In supravital preparations there may be various confusing cellular phenomena such as vacuolization, erythrophagocytosis, leukocytophagocytosis, nucleophagocytosis, or the Kurloff cell formation if fresh guinea pig cells are being observed. If a preparation is to show L.E. cells in the stained film, however, the living preparation also can be seen, by the trained observer, to contain these cells.

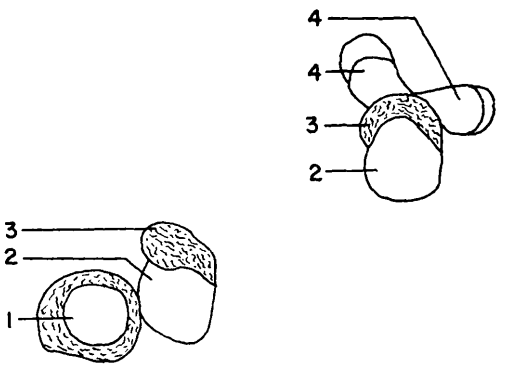

FIG. 2a. Diagram of Fig. 2.

1. L.E. cell 2. Chemotactic L.E. body
3. Phagocytic granulocyte 4. Distorted erythrocyte

TABLE I. Comparison of Bead Traumatization and Freezing Technics in Production of the L. E. Cell Phenomenon.

Patient's own cells						Tests
Positive	using	beads;	positive	using	freezing	11
Negative	"	" ;	negative	"	"	44
Positive	"	" ;	"	"	"	4
Negative	"	" ;	positive	"	"	0
Patient's serum and GP cells						
Positive	using	beads;	positive	using	freezing	11
Negative	"	" ;	negative	"	"	92
Positive	"	" ;	"	"	"	0
Negative	"	" ;	positive	"	"	5

The widely accepted technic utilizing rotating glass beads as the source of trauma(4) to produce this phenomenon was the control standard in the present study. Blood and sera from many normal individuals and from individuals with various infectious, neoplastic, immunologic, and idiopathic diseases were subjected both to the freezing technic and to the bead traumatization technic.

The L.E. cell phenomenon could be produced using the freezing technic only when the blood of the individual also exhibited, or had exhibited, the phenomenon using the bead technic. Table I summarizes the tests on single samples of blood or serum using both technics. In 22 instances positive tests were obtained using both traumatization and freezing technics. Using heparinized patient blood, 4 instances were found in which the test was positive using beads but negative using freezing. Using stored patient serum with guinea pig cells, however, 5 instances were found in which freezing brought out the phenomenon though the bead traumatization test was negative. In these last 5 instances, the blood of the patients had at other phases of the illness been demonstrated definitely positive using beads.

Stained films of frozen preparations from which the L.E. factor is absent, show many large finely reticulated or lacy, relatively amorphous nuclear remnants which are not attractive to, nor engulfed by, phagocytes. No L.E. cells are produced.

The use of cold injury to the leukocytes in producing this phenomenon provides the advantages of (a) a large yield of L.E. material and L.E. cells, (b) freely suspended L.E. material and L.E. cells in a liquid medium without large cellular aggregates, and (c) ability to maintain the L.E. material indefinitely at minus 50°C.

Summary. 1) Cold (minus 50°C) injury applied to leukocytes in the presence of the L.E. cell factor results in production of a large amount of L.E. material. This material usually exerts chemotaxis and, in the presence of fresh phagocytes at 37°C, allows the formation of great numbers of typical L.E. cells. In the absence of the L.E. cell factor, no L.E. material nor L.E. cells are formed. 2) The large mass of this unique material which can thus be produced now lends itself to analytic methods and experimental procedures not previously applied.

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