METHODS

Demonstration of the "L. E." Cell without Use of Anticoagulants

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HARGRAVES, in 1948, described a specific cellular abnormality, the "L. E." or lupus erythematosus cell, occurring in centrifuged heparinized bone marrow aspirates from patients with systemic lupus erythematosus. The "L. E." cells were predominantly polymorphonuclear neutrophilic leukocytes which had engulfed a large mass of amorphous material. The mass filled the cytoplasmic compartment of the granulocyte and pushed the nucleus to the margin of the cell. Haserick considered the arrangement of two or more granulocytes about a globule of identical material simulating a rosette to be part of the same phenomenon. Sundberg and Lick showed that "L. E." cells could be found in smears of the buffy coat obtained by centrifuging oxalated peripheral blood of patients with this disease.

The factors leading to the formation of the "L. E." phenomenon have been the subject of much investigation. Haserick and Hargraves and their associates demonstrated that a factor was present in the plasma of patients with systemic lupus erythematosus which would cause the development of the "L. E." phenomenon when added to cells from normal marrow or blood. Further investigations by Haserick have shown that the "blood factor" is in the gamma globulin fraction of the plasma proteins.

The visible changes in the leukocytes exposed to this factor have been a valuable aid in the diagnosis of systemic lupus erythematosus. They have usually been observed when special technics of preparation are employed, although Lee, Michael and Vural state that they found "L. E." cells in preparations made directly from clotted blood taken from patients with systemic lupus erythematosus. The relationship of the "L. E." cells and rosettes to the pathogenesis of this disease is still a matter of speculation. The possibility that these cells are artifacts induced by the anticoagulant or the concentrating technic has not been conclusively eliminated. Our investigation was planned to determine whether anticoagulants were required for the production of the "L. E." phenomenon.

The peripheral leukocytes of patients with systemic lupus erythematosus are in constant contact with plasma containing the "L. E." factor. The "L. E." cell should therefore be demonstrable in blood smears taken directly from these patients if anticoagulants do not play a role in their production. In an attempt...
to settle this point, we searched many unconcentrated direct smears from 2 patients with systemic lupus erythematosus. This proved to be an exhaustive task as would be expected from the rarity of "L. E." cells in highly concentrated preparations of peripheral leukocytes. To facilitate the study two methods of concentrating the leukocytes without anticoagulants were devised.

**Methods**

1. Twenty cc. of venous blood from patients with systemic lupus erythematosus were defibrinated by agitation in a small jar containing a bent paper clip. The defibrinated blood was transferred to a serologic tube and centrifuged for five minutes at 1800 r.p.m. The buffy coat was indistinct so the top of the cellular layer was pipetted off and recentrifuged in a Wintrobe hematocrit tube. Smears were made from the buffy coat thus obtained. Similar preparations of blood from 2 laboratory workers were examined as controls.

2. Blood from the same patients was drawn into a syringe coated with "silicone" and transferred to a serologic tube similarly coated. This was centrifuged at 1000 r.p.m. for two minutes and smears were made from material pipetted from the region of separation of the cells from the plasma.

**Results**

On the smears of the buffy coat of concentrated defibrinated blood from the patients with systemic lupus erythematosus the neutrophilic leukocytes were frequently grouped in large clusters of 10 to 50 cells. "L. E." cells were observed frequently in these clusters and also were found singly on the slides. Smears from the controls showed no "L. E." cells or clumping of the neutrophils. Serum obtained by defibrinating the blood from patients with systemic lupus erythematosus was mixed with an equal volume of concentrated leukocytes from normal defibrinated blood and centrifuged immediately. "L. E." cells and clustering were observed on smears of this preparation. "L. E." cells and rosettes were
observed in small numbers in the unmodified blood from patients with systemic lupus erythematosus concentrated in silicone coated tubes.

**Fig. 2.**—A clump of “L. E.” cells in the unmodified blood of a patient with systemic lupus erythematosus concentrated in “silicone” coated glassware.

**Fig. 3.**—Clumps of polymorphonuclear leukocytes in defibrinated blood from a patient with systemic lupus erythematosus.

These results indicate that the “L. E.” cell is not an artifact induced by anticoagulants. The role of mechanical agitation cannot be definitely evaluated from this study. Probably mechanical agitation is not necessary for the formation of
“L. E.” cells or rosettes as they were demonstrated in preparations in which agitation was minimal. We have since examined many direct smears of finger tip blood from these patients and found one neutrophilic leukocyte which appeared to be engulfing amorphous pink material. This single finding must be confirmed.

**SUMMARY**

“L. E.” cells and rosettes were demonstrated in the blood of patients with systemic lupus erythematosus without the use of anticoagulants. Concentration technics using defibrinated blood and unmodified blood in silicone coated tubes were employed.

**REFERENCES**

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