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foie, l'alcool en particulier; proscription soigneuse de toutes les substances que nous avons énumérées susceptibles de déclencher une poussée. La diététique ne semble pas jouer un grand rôle dans le déterminisme des accidents depuis que l'on sait que l'organisme synthétise les porphyrines à partir du glycocolle et de l'acide acétique et qu'il est impossible de créer une carence en glycocolle, que l'organisme fabrique indépendamment de tout régime.

Le traitement des accidents est remarquablement décevant. Les extraits hépatiques, l'acide folique, la vitamine E, la vitamine PP, la vitamine B_1 ont été prônés; l'ACTH a été tenté sans résultat.

Contre les crises abdominales douloureuses, l'atropine, la morphine elle-même se montrent inefficaces. Dans un cas l'association scopolamine-morphine nous a paru apporter une sédation. Wehrmacher a proposé le blocage des splanchniques par la novocaine et utilisé le tetraethylammonium, le chlorure de tubocurarine, le priscol. Pour parer à la déshydration liée aux vomissements, des injections de sérum physiologique seront indiquées. Contre les troubles respiratoires enfin, la mise en poumon d'acier est une indication absolue. En cas de crises convulsives, il conviendra de s'abstenir des barbituriques et de donner la préférence aux bromures.

THE L.E. (LUPUS ERYTHEMATOSUS) CELL REACTION*

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THE L.E. (LUPUS ERYTHEMATOSUS) cell reaction, as commonly performed, is an *in vitro* phenomenon which may be observed in patients with acute disseminated lupus erythematosus when plasma, serum or serous effusions are permitted to remain in contact with a mixture of freshly drawn white cell elements of the blood or bone marrow. The phenomenon was first reported in 1948 by Hargraves, Richmond and Morton,[1] who observed the reaction in heparinized preparations of bone marrow which had been allowed to stand for a time before being transferred to glass slides for staining.

Since its introduction the test has been the subject of much investigation, dealing mainly with different methods of reproducing or enhancing the phenomenon, simplification of the procedure, and identification of the factor responsible for its occurrence.[1-39] Whatever method is employed, it is desirable to obtain a good concentration of cells in order to facilitate examination of the stained smear (Table I). Heparinized bone marrow yields excellent results, but marrow aspiration may be a distressing procedure in patients who are seriously ill. Fortunately it is not essential for the production of the phenomenon since venous blood serves equally well. The blood may be defibrinated or anticoagulants may be added but they are not necessary. Anticoagulants do not enhance the formation of L.E. cells and may actually reduce the intensity of the reaction.[23, 30, 40]

TABLE I.

L.E. CELL PHENOMENON—METHODS

Heparinized bone marrow
Heparinized venous blood
Oxalated venous blood
Defibrinated venous blood
Clotted venous blood
L.E. factor and donor cells

In lieu of using the subject's whole blood or bone marrow (Table II), the test may be performed by mixing the patient's plasma, serum or serous effusions as a source of L.E. factor, with bone marrow from a donor subject or

TABLE II.

L.E. CELL PHENOMENON—PARTICIPATING FACTORS

L.E. factor	*Living cells*
Plasma	Human bone marrow
Serum	Animal bone marrow
Serous effusions	Buffy coat, venous blood
Joint effusions	Buffy coat, myel. leukæmic blood
C.S.F. and urine (rarely)	Joint effusion cells

Role of platelets—activation of L.E. factor?
Temperature — 22° -37° C.
Time interval — ½ - 2 hours.

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various laboratory animals. As a substitute for bone marrow, cells may be added from peripheral blood buffy coat or from joint effusions[41] of other patients. Perhaps the simplest and most effective method, as a laboratory procedure, is the use of untreated venous blood which has been permitted to clot.[5, 23, 30] After one or two hours, the serum is removed and the residual clot macerated with applicator sticks or passed through a wire mesh screen. The cells expressed from the clot are placed in a Wintrobe hæmatocrit tube and centrifuged at high speed (2,000 r.p.m.) for 5 minutes. The buffy coat is then carefully removed with a fine glass pipette and smeared on glass slides. Any of the stains for routine blood films are satisfactory.

The optimum period of time that the clotted specimen of blood should stand before the smears are prepared varies greatly. Twenty minutes may be adequate, but longer periods up to 2 hours have been advocated. Where the test is not strongly positive, 20 minutes may not be sufficient to permit the formation of well-developed L.E. cells. Conversely, where the test is strongly positive, 2 hours may be too long as most of the cells in the stained smear may be smudged or poorly preserved. The process is hastened by incubation at 37° C., but this is not essential. Room temperature (22° C.) is quite satisfactory, although further cooling tends to inhibit the phagocytosis which is essential for the production of the typical cells.

The factor responsible for the phenomenon resides within the plasma, serum and tissue fluids of the patient. Positive tests have been obtained with joint effusions,[41] pleural effusions[42] and pericardial effusions,[43] and have been reported with cerebrospinal fluid[44] and urine.[45, 46] There appears to be no inherent abnormality in the leukocytes mixed with the test serum, since they merely provide the cells necessary for the reaction. The phenomenon is dependent upon the L.E. factor acting in the presence of living leukocytes but is independent of the source of the latter, although differences in susceptibility of leukocytes from varying human and animal sources have been observed.[47] The principle of mixing the test serum with cells from another source has been criticized on the basis that additional factors may be introduced.[34] A similar criticism may be made of the practice of adding dead cells, damaged nuclei or nucleoprotein material from other sources to enhance the formation of L.E. cells.[37-39] Observations on electrophoretically separated serum proteins and immunological studies indicate that the L.E. factor (Table III) is contained in the gamma

TABLE III.

L.E. FACTOR—PROPERTIES

L.E. factor	—present in gamma globulin
Preservation	—sterile, frozen
Inactivation	—bacterial contamination, heating to 65° C., L.E. antibodies, desoxyribonuclease inhibitor, para-aminobenzoic acid.
Passive transfer—to animals and fetus	

globulin fraction.[22, 48, 49] It is quite stable in serum when sterile, and may be preserved for one year or longer in the frozen state,[41] but is inactivated by heating to 65° C. or by bacterial contamination.[49] It has been reported to be inhibited by the addition of para-aminobenzoic acid and by L.E. antibodies developed in rabbits against the specific gamma globulin, but not by cortisone, testosterone, œstradiol, progesterone or control antibodies developed in rabbits against normal human plasma.[13, 49, 50] The L.E. factor can be transferred passively to laboratory animals[51] and may pass through the placental barrier,[52] although it need not be associated with symptoms of lupus erythematosus in the infant.

It has been suggested that the reaction may be initiated by the breakdown of platelets.[3] This speculation was based on the observation that the phenomenon did not occur *in vivo*, and also on the occurrence of symptoms of acute disseminated lupus erythematosus with positive L.E. tests in three patients after splenectomy for hypersplenism. Thrombocytopenia had been a feature in two of the patients and neutropenia in the other. Further confirmation of this role of the platelets has recently been reported, stressing the importance of blood coagulation in accentuating the reaction.[30, 40] On rare occasions, L.E. cells have been found in freshly drawn blood smears[26, 53] and in sections of tissue obtained at autopsy.[32, 54] It is possible that L.E. cells can form *in vivo* although they may be removed from the circulation by reticulo-endothelial cells. *In vivo* production of L.E. cells in small numbers can be demonstrated by the application of a rubber tourniquet around the finger for 30 minutes before doing a needle puncture,[41] but the fact that they cannot usually be demonstrated in fresh smears of capillary blood, when the test is strongly positive *in vitro*, suggests that they are not formed in any large numbers.

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Two structures are regarded as characteristic of this phenomenon: (1) the L.E. cell, and (2) the rosette. To this may be added a third, viz., the extracellular, amorphous, nuclear masses. The typical L.E. cell[1] has been described as a mature polymorphonuclear neutrophil, containing an amorphous and structureless inclusion body, which takes on nuclear staining characteristics and which displaces the segmented neutrophil nucleus to one side (Fig. 2c, d). Originally the inclusion body was regarded as representing either phagocytized nuclear material from other cells in which the chromatin structure had undergone partial digestion, or alternatively, autolysis of one or more lobes of the neutrophil of the involved cell. In positive smears, amorphous masses similar to the inclusion bodies in the L.E. cells, were frequently found extracellularly. The so-called rosette (Fig. 6a) has been described as a central mass of amorphous nuclear debris, surrounded by a collar of neutrophilic leukocytes. While it is a significant part of the reaction it is probably less specific than the L.E. cell.

Following Hargraves's original report, it was suggested that the inclusion bodies might represent precipitated protein, or that they might be of megakaryocyte or platelet origin.[48] However, it is now accepted that they are of nuclear origin, and differential staining techniques with Feulgen and methyl green have shown the desoxyribosenucleic acid to be largely in the depolymerized state.[1, 24, 54] There has been some doubt whether this material is derived primarily from lymphocyte or neutrophil nuclei, but it is probable that they arise from both,[22] or perhaps even from nuclei of any type, which are then ingested by actively phagocytic cells. The parent L.E. cell was initially described as being a mature neutrophil, but it has since been shown that the extruded nuclear material may be phagocytized by band forms, eosinophils, monocytes, lymphocytes, and by myelocytes.[5, 22, 24, 32, 34, 41]

METHODS

The present study was undertaken to determine the morphology of the typical L.E. cells and rosettes, the stages in the development of these structures, participation of various cells of the leukocytic series in the reaction, correlation to the severity of symptoms and response to treatment, and the diagnostic significance of the test as related to disseminated lupus erythematosus.

The test was performed by most of the methods which have been described. These included the use of the patient's heparinized or oxalated bone marrow; patient's heparinized or oxalated venous blood; patient's defibrinated blood; patient's serum or plasma mixed with peripheral blood buffy coat or bone marrow from donor sources or with cells from joint effusions; patient's pleural or joint effusions mixed with peripheral blood buffy coat or bone marrow from donor sources; and simply clotted venous blood. Because of its simplicity and consistent results most of the tests were done with clotted venous blood which had stood at 21° or 37° C. for two hours. The period of incubation was often shortened in the case of tests which were strongly positive, varying from 10 minutes to two hours. The observations were supplemented by wet film preparations studied by phase contrast microscopy and recorded by micro-cinematography.

The first 1,250 tests comprising a total of 750 patients suffering from a variety of diseases have been reviewed. These included disseminated lupus erythematosus and related disorders of the collagen group, varying types of bone marrow suppression, blood dyscrasias, hypersensitive states, essential hypertension, renal disease, chronic hepatitis, other disorders associated with hyperglobulinæmia and a variety of miscellaneous conditions. The smears were prepared with Wright's blood stain and were examined individually by the author. The photographs illustrating the observed cellular changes were prepared from patients suffering from disseminated lupus erythematosus. Changes of a similar character observed in other diseases will be described in a separate report.

OBSERVATIONS

Formation of L.E. cells and rosettes.—While information has accumulated concerning the source of the nuclear material which forms the inclusion body in the L.E. cell, a study of the reaction in wet film preparations as well as fixed stained smears, serially prepared at short time intervals, provides clear evidence on the sequence of events. These observations corroborate the reports of other investigators.[1, 20-24, 34]

The time relationship of the changes varies considerably in different preparations depending

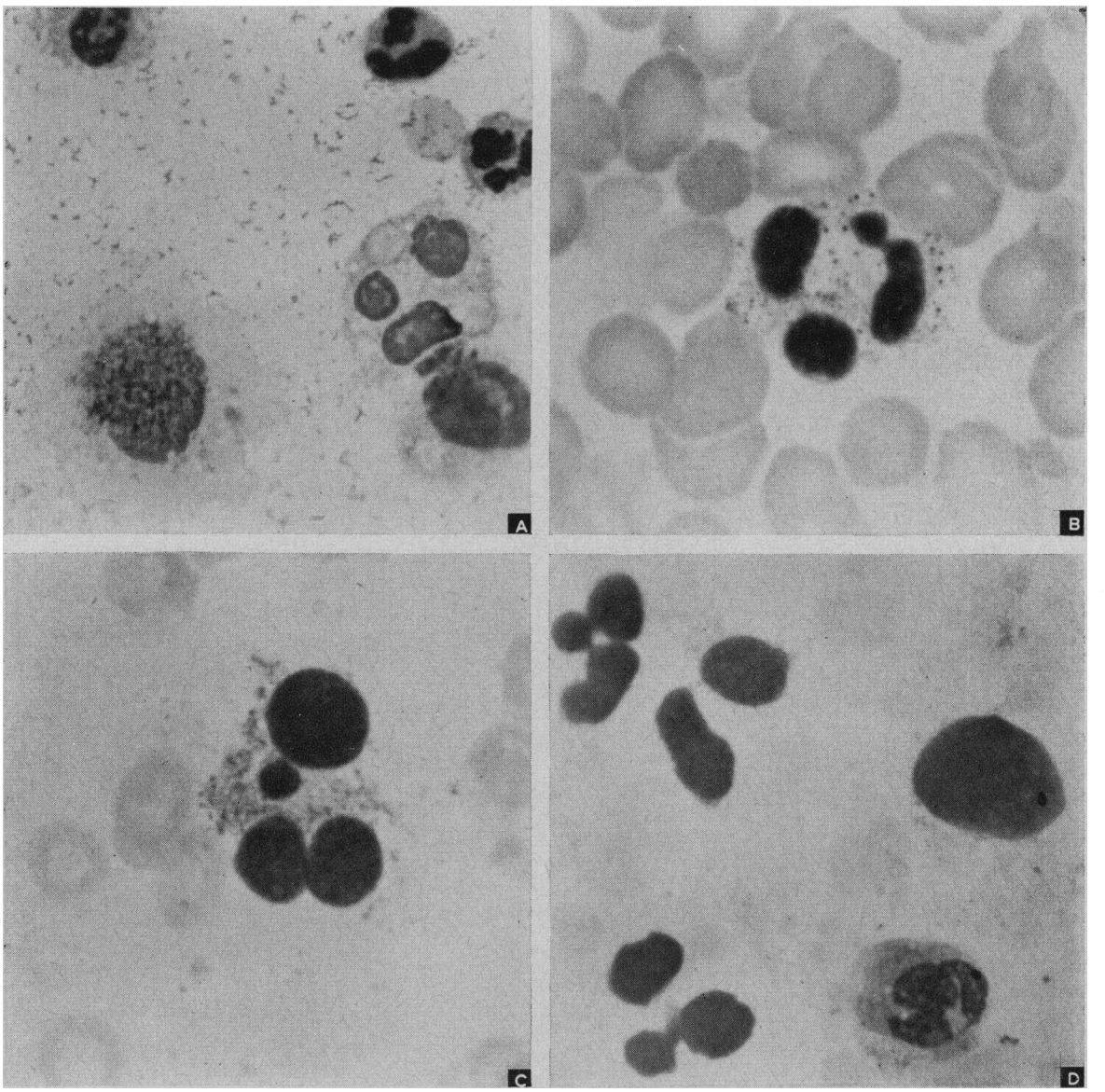

Fig. 1.—Formation of the amorphous nuclear bodies: (a) swelling of neutrophil and lymphocyte, (b) segments of neutrophil nucleus becoming homogeneous in appearance, (c) rupture of cell membrane with a few granules remaining and (d) free, amorphous, nuclear bodies originating from neutrophils and lymphocytes. × 1500.

on the intensity of the reaction; when strongly positive, the latter may be observed within 10 minutes of drawing the blood. The earliest recognizable change represents a degeneration of some of the neutrophils, lymphocytes and possibly other cells, with an alteration in their staining characteristics. The nuclei become larger and stain less intensely, and the chromatin structure becomes less distinct (Fig. 1a). Within a short period the nuclei of the affected cells become homogeneous in appearance with a loss of all chromatin detail (Fig. 1b). The cell membranes then rupture (Fig. 1c), releasing amorphous nuclear segments of different sizes. The free nuclear masses thus released may remain in small clusters, characteristic of the grouping of polymorphonuclear nuclei, and may be connected by thin filaments of chromatin (Fig. 1d) or surrounded by granules (Fig. 1c). Sometimes this change, occurring in a variable number of cells, may be all that is evident, especially when the time interval has been too short. In positive smears such amorphous masses can usually be found lying extracellularly as well as in the form of intracellular inclusions. They vary in size, shape and staining properties and, while they are usually homogeneous, they may sometimes appear granular. Their presence extracellularly should arouse suspicion that the smear will contain L.E. cells.

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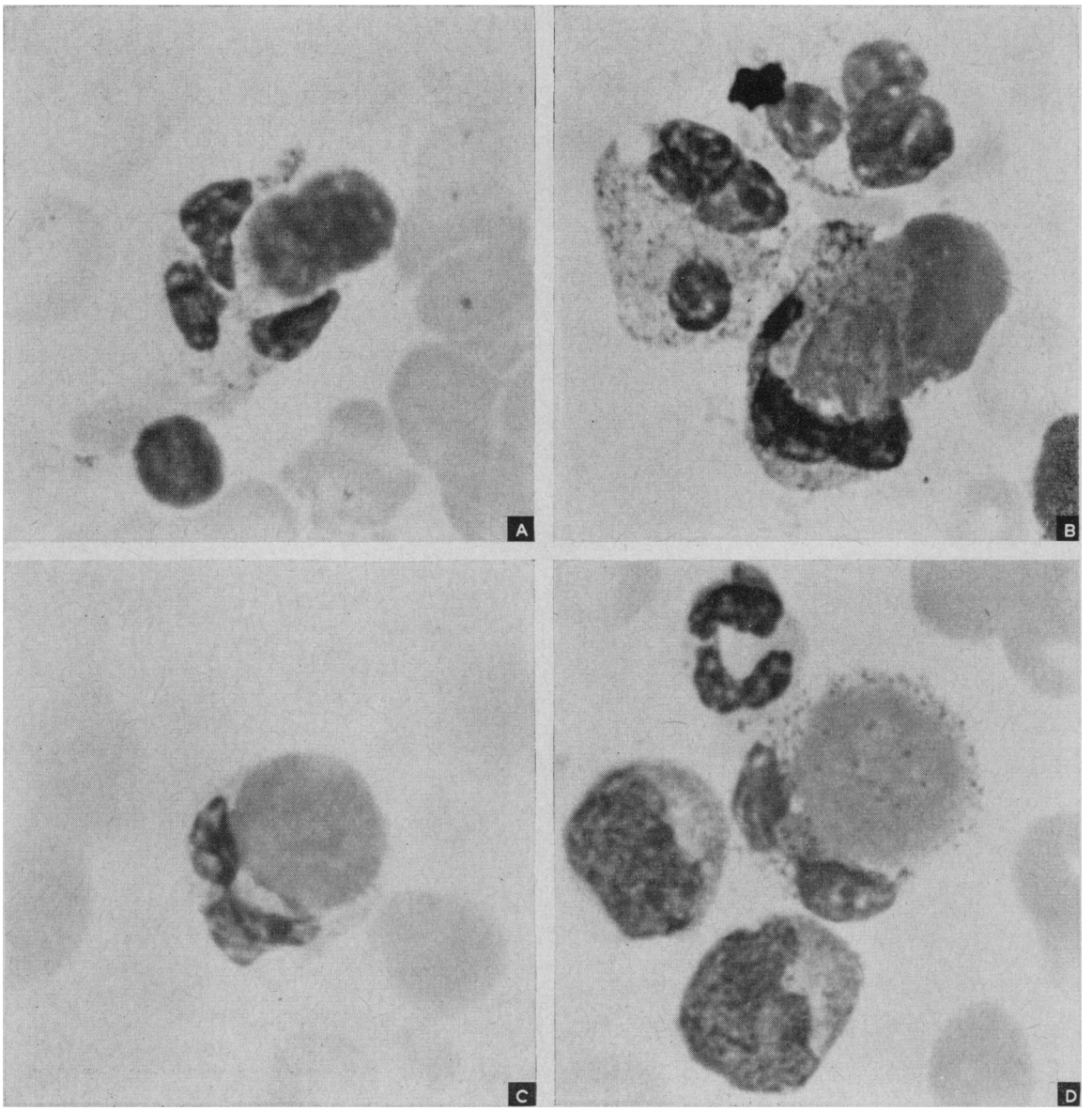

Fig. 2.—Formation of L.E. cells: (a) and (b) neutrophils engulfing free nuclear masses; (c) and (d) fully formed L.E. cells; mature neutrophils containing an amorphous inclusion body in their cytoplasm, displacing the parent nuclei to one side. × 1500.

In stained smears the amorphous bodies can often be seen in various stages of phagocytosis by other surviving neutrophils (Fig. 2a, b). When fully enclosed they present the appearance of the typical L.E. cell with the ingested mass completely surrounded by cytoplasm and forming an inclusion body (Fig. 2c, d). The characteristic cells, often grouped in clumps, are easily recognized although they may be distorted and misshapen (Fig. 3). While the typical L.E. cell contains a single inclusion body, some cells contain two or more inclusion bodies (Fig. 4a, b, c), probably representing the multiple nuclear lobes of destroyed neutrophils. Most of the nuclear masses appeared to be derived from

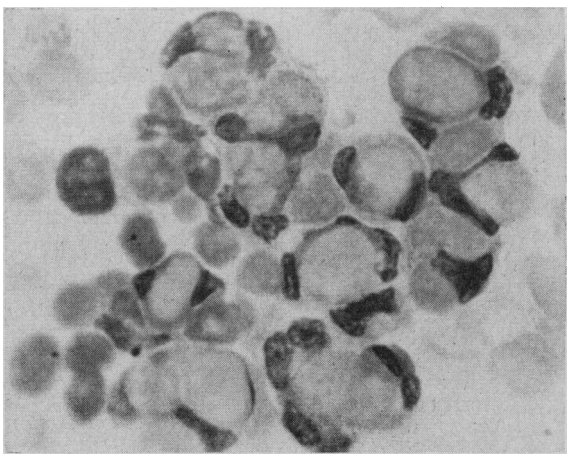

Fig. 3.—Large clump of L.E. cells containing varying numbers of inclusion bodies. Amorphous nuclear material is also present extracellularly. × 1000.

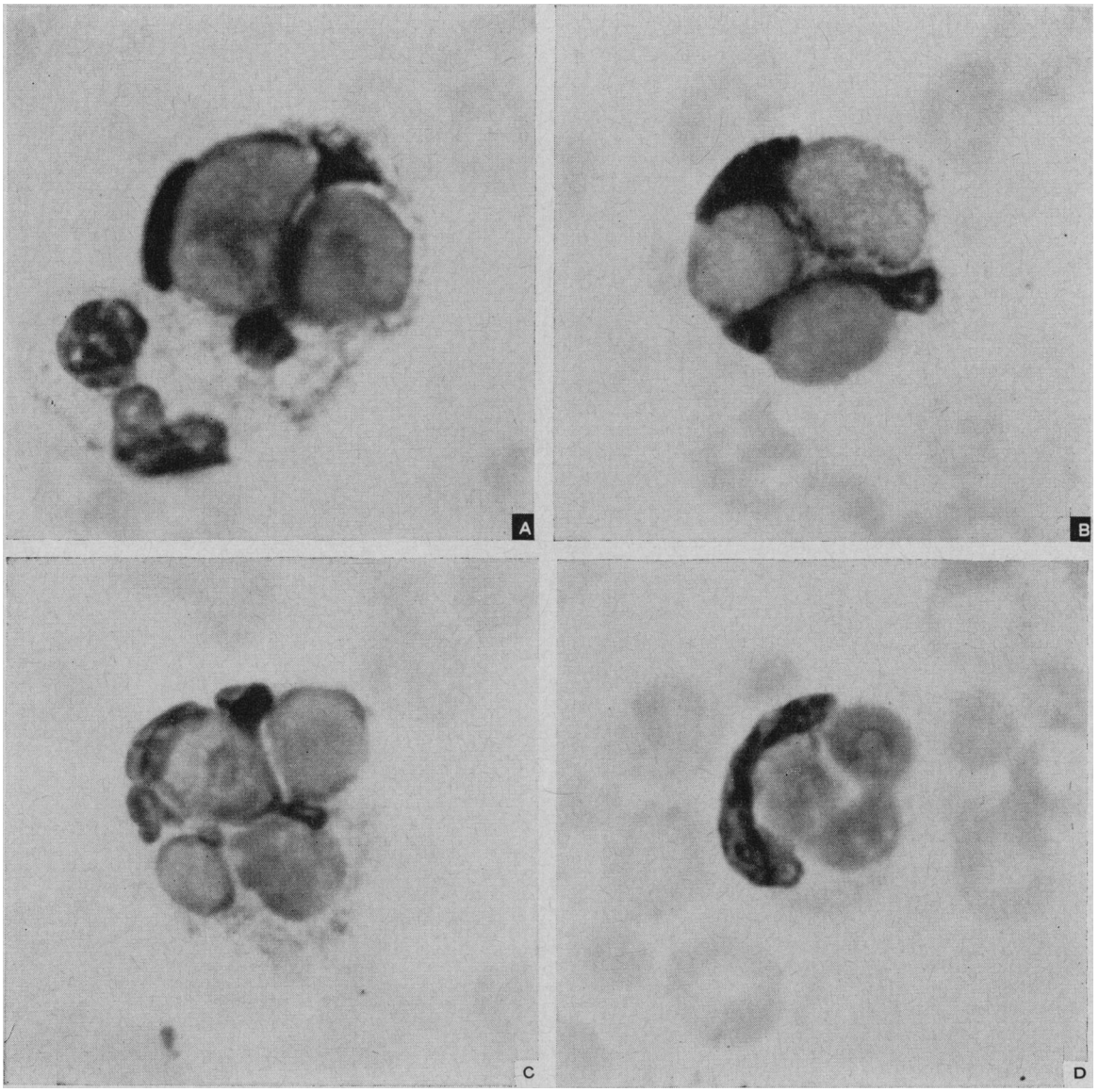

Fig. 4.—L.E. cells containing (a) two, (b) three and (c) four inclusion bodies; (d) an inclusion body derived from an incompletely segmented band form. × 1500.

mature segmented neutrophils but some may be recognized as having originated from lymphocytes or band forms (Figs. 1d, 4d). In the formation of the rosette, phagocytosis of the free nuclear material may be attempted by two, three or four leukocytes (Fig. 5a, b, c, d). When it is surrounded by a collar of cells, it presents the appearance of a rosette (Fig. 6a).

The above sequence of events in the formation of L.E. cells has been confirmed in wet film preparations and has been recorded with time-lapse micro-cinematography.[41]

Participation of other leukocytes.—In earlier reports, the parent cell which ingested the free nuclear material was described as a mature neutrophil. They usually make up the bulk of the leukocytes in the circulating blood and tend to be the most actively phagocytic. Under suitable circumstances, however, other cells may take part in the reaction. Nuclear swelling and cell lysis have been observed to affect neutrophils, lymphocytes and eosinophils,[41] though neutrophils are principally affected. When suitable donor cells are supplemented to add greater numbers of eosinophils, lymphocytes, monocytes, plasma cells or myelocytes, these too may be phagocytic and will sometimes ingest the nuclear bodies to form parent L.E. cells.[41] By this technique it has been possible to show that most of the cells of the leukocytic series in the blood may take part in the reaction.

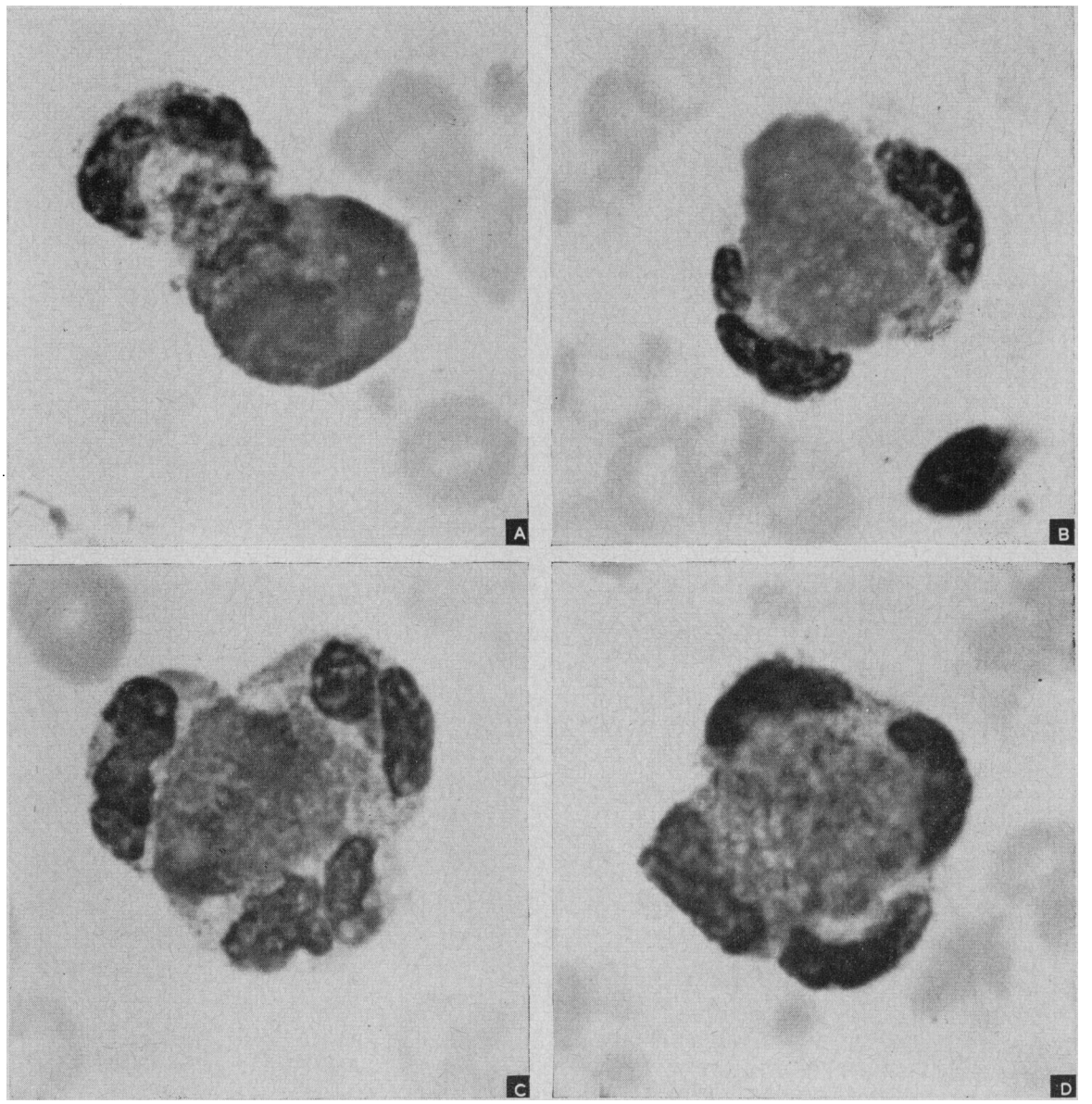

Fig. 5.—Formation of rosette. Phagocytosis of amorphous nuclear material attempted by (a) one, (b) two, (c) three and (d) four neutrophils. × 1500.

Interpretation of the L.E. test.—The recognition of a positive test presents no problem when many L.E. cells are found (2-30% of the neutrophil count). Since the intensity of the reaction is variable in different tests, difficulty may sometimes be encountered, especially where typical cells are present but few in number (less than 1%), or where a large proportion of the intracellular inclusion bodies, even though numerous, are of a more granular type (Fig. 7b, c). On rare occasions, only the first phase of the reaction may be evident, consisting of lysis of many cells with release of their amorphous nuclear fragments (Fig. 1c, d). The phagocytic stimulus may not be prominent or may be entirely lacking, so that few or none of the nuclear bodies are ingested by surviving cells. Such changes are probably significant even in the absence of phagocytosis but at present cannot be interpreted as a positive test. Repetition of the test on other occasions may sometimes yield typical L.E. cells. This raises the possibility of separate factors being responsible for the cell destruction and the phagocytosis. A search should always be made for the extracellular nuclear material in addition to the intracellular inclusion bodies. It provides evidence of the so-called nucleolysis, which is fundamental to the reaction. Even so, doubtful smears will be

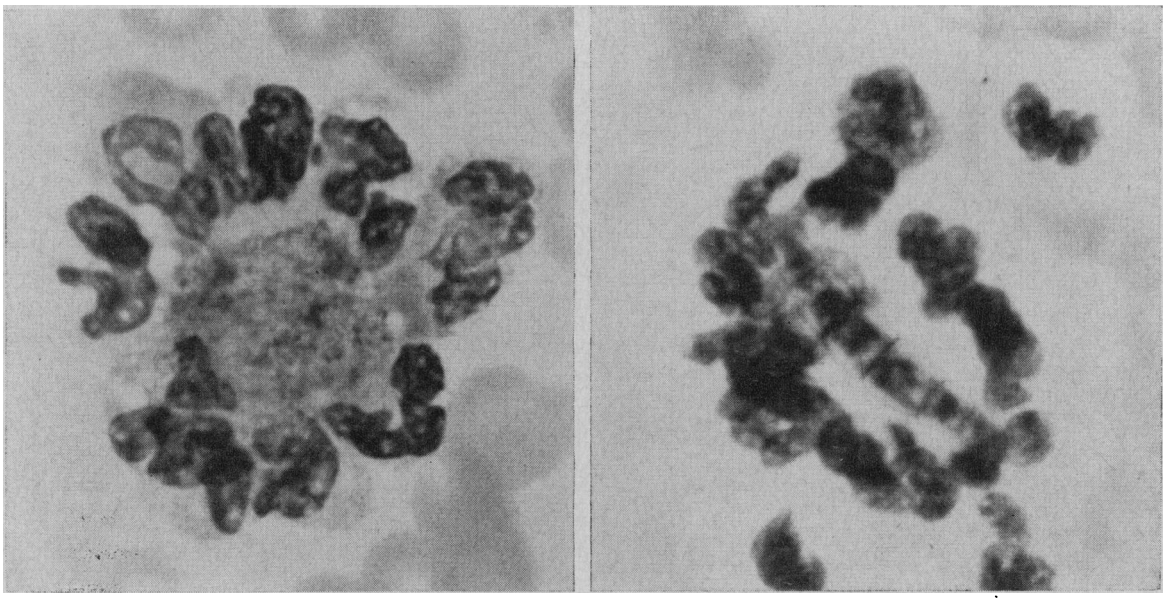

Fig. 6.—(a) Typical "rosette". A collar of neutrophils surrounds a mass of amorphous nuclear debris. The granularity of the nuclear material in the centre is due to overlying cytoplasmic granules. (b) Rosette-like formation of neutrophils around a foreign body. × 1000.

encountered, a point which has not been emphasized in the literature.

The quality of the preparation and the interpretation of the result will necessarily be governed by the technical skill and experience of the observer. Where doubt exists, the test should be repeated under carefully controlled conditions, preferably at 37° C. rather than room temperature, and permitted to stand the full two hours. It is not desirable to extend the incubation period beyond two hours. If the interpretation is still uncertain, it should be reported as doubtful. The reaction is not one of precision and at best is a crude method of demonstrating the presence of an abnormal plasma constituent which has an injurious effect on the nucleated white cells.

Leukocytes which contain ingested lymphocyte or polymorphonuclear nuclei (so-called secondary nuclei), in which the chromatin pattern remains relatively intact, do not constitute L.E. cells and have been named "tart cells" (Fig. 7a). They occur in a variety of circumstances and even in the absence of any disease state. However, "tart cells" may also be found in disseminated lupus erythematosus, along with intermediate forms containing granular inclusion bodies and typical L.E. cells (Fig. 7a, b, c, d), with amorphous inclusion bodies. Neutrophils containing ingested red cells,[35] precipitated protein material[55] or fungus forms,[56] likewise do not constitute L.E. cells and may be a source of error.

There is little uniformity of opinion as to the number of L.E. cells necessary for a positive test. Lacking quantitative criteria, the interpretation of results has depended to a large extent on the judgment of the examiner. In the eyes of some observers, one typical L.E. cell constitutes a positive test, but the dangers of a "one-cell diagnosis" have been stressed.[4] Others have recommended a percentage count per 1,000 neutrophils.[22, 75] In the present study a smear was considered positive when 10 or more characteristic L.E. cells could be found during a 15-minute search, associated with the presence of extracellular, amorphous, nuclear masses. The vast majority of positive tests contained numerous L.E. cells, varying from 2 to 30% of the neutrophil count.

Results.—The following observations are based on a survey of 750 patients with a variety of diseases and comprising a total of 1,250 tests (Table IV). In patients with disseminated lupus erythematosus the test was repeated on frequent occasions during the course of the illness and follow-up period, regardless of whether the first test was positive or negative. Repeated tests were also done in certain other diseases, particularly when the initial test was either positive or doubtful, or when the course of the illness or changes in the serum protein pattern suggested that the reaction might later be

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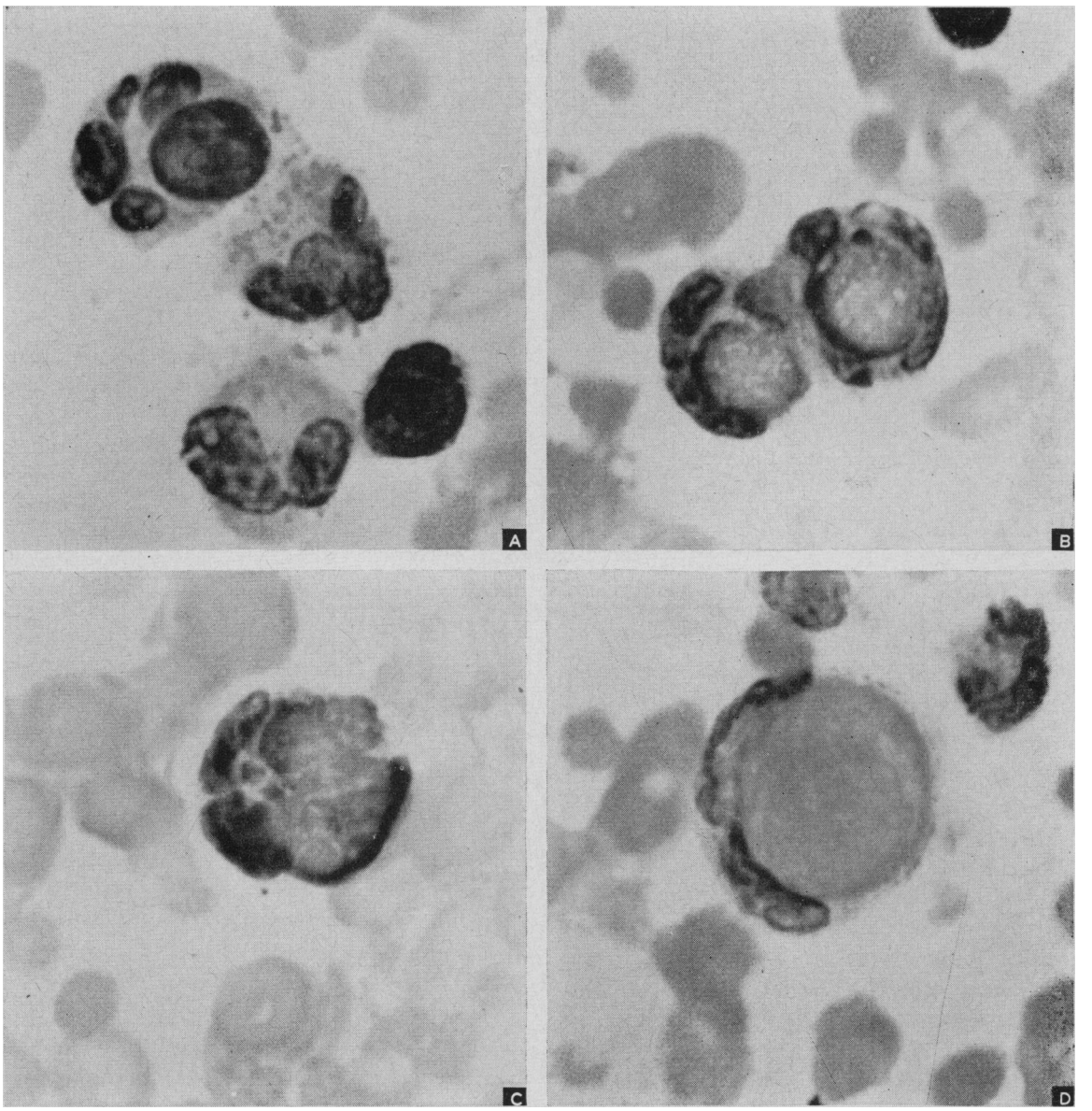

Fig. 7.—(a) Neutrophil containing a "secondary" lymphocytic nucleus (tart cell), (b) and (c) neutrophils containing granular inclusion bodies, and (d) typical L.E. cell with large, homogeneous inclusion body. All forms were found in one smear from a patient with acute disseminated lupus erythematosus. × 1500.

demonstrated. Where these conditions did not exist and the initial test was negative, it was not repeated.

The L.E. phenomenon was consistently demonstrated in 38 of 41 patients with an acceptable diagnosis, either clinically or pathologically, of acute disseminated lupus erythematosus, but could not be demonstrated in three others. L.E. cells were not found in seven patients with subacute disseminated lupus erythematosus or in six patients with chronic discoid lupus. In other members of the collagen group of diseases, the phenomenon was present in only one patient each with dermatomyositis,* polyarteritis nodosa† and scleroderma.‡ Of greater interest, however, was the fact that positive tests were found in 17 of 180 patients with chronic rheumatoid arthritis. All had classical disease of 3 to 31 years' duration, with gross joint deformities, radiological evidence of articular destruction and associated juxta-articular, subcutaneous rheumatoid nodules in most instances. A number of these patients who have since been autopsied did not show the lesions of lupus erythematosus.

*Confirmed by muscle biopsy.
†Confirmed at autopsy.
‡Courtesy of Dr. R. Volpe and Dr. J. T. Hauch.

TABLE IV.

Disease	No. of patients		No. of tests		
	Positive	Negative	Positive	Negative	Doubtful
Acute diss. lupus eryth.	38	3	186	47	2
Subac. diss. lupus eryth.	0	7	0	18	0
Chr. discoid lupus eryth.	0	6	0	6	0
Dermatomyositis	1	6	1	8	2
Polyarteritis	1	12	3	23	0
Scleroderma	1	20	1	32	0
Undiff. collagen disease	0	17	0	21	0
Rheumatoid arthritis	17	163	43	255	18
Ankylosing spondylitis	0	13	0	14	0
Rheumatic fever	0	18	0	21	0
Reiter's syndrome	0	3	0	4	0
Gout	0	4	0	5	0
Cirrhosis (all types)	3	13	11	15	1
Nephritis (all types)	0	18	0	20	0
Essential hypertension (excluding Apresoline reactions)	0	21	0	23	0
Dermatoses	0	15	0	15	0
Erythema nodosum	0	4	0	4	0
Chr. ulcerative colitis	0	2	0	3	0
Sarcoidosis	0	4	0	4	0
Cryoglobulinæmia	0	3	0	9	0
Myeloma	0	8	0	12	0
Lymphomas	1	10	1	17	0
Leukæmias	0	5	0	5	0
Acq. hæmolytic anæmia	1	11	1	12	0
Idiopathic thrombocytopenia	0	12	0	23	0
Pancytopenia	0	2	0	2	0
Aplastic anæmia	0	4	0	4	0
Serum reactions	0	11	0	12	0
Gold reactions	0	4	0	7	0
Penicillin reactions	0	10	0	14	0
Phenylbutazone reactions	2	1	4	15	4
Apresoline reactions	4	1	9	11	6
Other drug reactions	0	3	0	3	0
Miscellaneous diseases	0	247	0	273	0
Total	69	681	260	957	33

L.E. cells were demonstrated in three patients with chronic hepatitis and evidence of severe liver damage, of undetermined etiology.[95] Two were young women with recurring jaundice of one to two years' duration, enlargement of the liver and spleen, ascites, spider telangiectases, impaired liver function and greatly increased levels of gamma globulin. The third, in whom the diagnosis remains in doubt, was a young female child with associated ulcerative colitis, chronic liver disease, ascites and transient effusions into the knees. One patient with Hodgkin's disease* and one patient with acquired hæmolytic anæmia of undetermined cause also exhibited the phenomenon.

Most reactions of the hypersensitivity type to a variety of therapeutic agents were not accompanied by a positive L.E. test. The phenomenon was demonstrated, however, in two patients after reactions to phenylbutazone. In one instance this was complicated by a hepatitis and necrotic bleeding ulcers of the trunk and legs, tending to heal slowly with much scarring. This patient now has evidence of chronic liver damage. The test was also positive in four patients with essential hypertension who suffered reactions to prolonged hydralazine therapy. Autopsy examination in one of the latter patients, after rupture of a dissecting aneurysm of the aorta a few weeks later, failed to disclose any lesions of lupus erythematosus. With the exception of the one patient who died, the L.E. test later became negative in all instances after the sensitizing agent had been withdrawn and the acute symptoms had subsided.

Correlation with severity of disease in disseminated lupus erythematosus.—From an examination of repeated preparations in patients with acute disseminated lupus erythematosus, it was apparent that the intensity of the reaction would vary in different subjects and also at different

*Confirmed at autopsy.

times in the same patient, irrespective of treatment. Although it could not be correlated with the severity of the illness in many patients, in a few instances it seemed to parallel the clinical course, being more difficult to demonstrate as symptoms improved. A positive test reverted to negative in one patient during an apparent complete remission following steroid therapy and has remained negative for more than two years after cessation of treatment. The test has become negative in four additional patients from one to two years after the induction of sustained but incomplete remissions with prolonged steroid therapy. Other patients have continued to show strongly positive tests regardless of the degree of clinical improvement.

While these patients frequently had a leukopenia, in general there was no correlation between the intensity of the reaction and the level of the white cell count. Greater difficulty was often experienced in obtaining a good concentration of cells in patients with a severe leukopenia, but this could be overcome by the addition of donor cells. In this regard it was of interest that cells obtained from patients with rheumatoid arthritis appeared to be more susceptible than cells from normal subjects.

DISCUSSION

Nature of the L.E. phenomenon.—The most significant feature of the L.E. cell reaction, as it occurs in freshly drawn peripheral blood or bone marrow, appears to be an unusual type of degeneration of some of the leukocytes, with swelling, lysis and release of their altered nuclei. The cellular damage which affects principally mature neutrophils, but also lymphocytes and possibly other cells, results from the action on the living cells of a toxic factor present in the plasma or serum. Since it was originally demonstrated in acute disseminated lupus erythematosus, it has been named the L.E. factor. Apart from evidence that it is contained in the gamma globulin fraction of the serum proteins, the exact nature and source of the factor is not known, nor is it certain that it represents a single substance. Its action is that of a lysin towards leukocytes. An activating mechanism may have to be invoked to explain the virtual lack of its occurrence *in vivo* as contrasted with the ease of its demonstration *in vitro*. Whether the sera of normal subjects also contain this factor, but suppressed by an inhibitor mechanism, still remains to be evaluated. On the basis of clinical observations, there is some suggestion that the phenomenon may be related to an abnormal immune mechanism or hypersensitive state.[12, 20, 61-65]

The second phase of the reaction, exemplified in the phagocytosis of the free nuclear masses, may be an integral part of the same phenomenon. It may also represent a normal response of leukocytes to the presence of foreign material, comparable to the ingestion of bacteria, yeast cells, etc. The rosette formation too would seem to represent a normal response of leukocytes in the exclusion of foreign material (Fig. 6b).

Thus far, the L.E. factor has not been shown to be responsible for the tissue changes occurring in disseminated lupus erythematosus, although changes have been described which closely resemble the L.E. phenomenon. Klemperer[66, 67] has presented evidence to show that the nuclei of mesenchymal tissue cells may undergo a similar type of necrosis in disseminated lupus erythematosus, resulting in the formation of basophilic, amorphous masses in the tissues called "hæmatoxylin bodies". It is probable that these represent a change comparable to the L.E. phenomenon.

Specificity.—Opinion regarding the specificity of this reaction appears to be divided. Many investigators consider the phenomenon specific for acute disseminated lupus erythematosus,[33, 38, 68-72] although admitting that it cannot always be demonstrated in the acute and subacute forms of the disease, and rarely if at all in the chronic form. Studies in which the test has been used as an absolute criterion for the diagnosis of lupus erythematosus, however, cannot be regarded as an impartial assessment. Others have taken the view that the test does not constitute an absolute diagnostic criterion,[22, 28, 73-75] but that it is a valuable aid in the diagnosis. This is supported by more recent evidence which indicates that the reaction may not be entirely specific. At the time of reporting, Hargraves *et al.*[1] stated that they were unable to demonstrate this phenomenon in all patients with acute disseminated lupus erythematosus, and acknowledged that the diagnosis might be questioned clinically in some of the 25 patients observed with a positive test. Different investigators have observed positive reactions in a variety of unrelated diseases, suggesting that the test may represent

an abnormal enzyme reaction,[23] or that it may be positive in circumstances where there is active destruction of body tissue,[1] disturbances of the antibody-producing mechanism[58] or related to hypersensitivity without exhibiting manifestations of systemic lupus erythematosus.[61] Among the disorders in which L.E. cells have been reported, though sometimes in small numbers, are leukæmia,[12] pernicious anæmia in relapse,[22] dermatitis herpetiformis,[22] acquired hæmolytic anæmia,[23] a type of chronic hepatitis (cirrhosis),[57-60] penicillin reactions,[61, 62] multiple myeloma,[77] moniliasis,[78] miliary tuberculosis,[79] periarteritis nodosa[80] and scleroderma.[81] Apart from disseminated lupus erythematosus, perhaps the highest incidence of positive tests has been reported in chronic rheumatoid arthritis.[74, 82-84] In some instances it has appeared to become positive during the severe relapse that has followed withdrawal of cortisone therapy.[83] It has been suggested by some that systemic lupus erythematosus may have been co-existent in these patients,[69, 70] but this would seem unlikely in all instances. Structures similar to L.E. cells have been produced with leukocytic antiserum,[63] desoxyribonuclease,[85] polyvinyl alcohol polysulfonic acid ester,[86] virus cultures recovered from patients with lupus erythematosus[87] and following prolonged administration of hydralazine therapy[88-92] in hypertension. Attempts to demonstrate the participation of serum desoxyribonuclease in the L.E. phenomenon have not been successful,[93] although a nuclease inhibitor derived from normal human leukocytes has been shown to inhibit the phenomenon.[94]

The test represents a type of serological reaction, possibly resulting from the production of auto-antibodies to leukocytes, and as such it would be reasonable to expect that it will have a degree of specificity comparable to that of other serological reactions. The demonstration of a positive test may be interpreted as providing corroborative support to the diagnosis of disseminated lupus erythematosus in patients with the symptomatology of that disease, and in this sense it undoubtedly is a valuable aid. It should, however, be interpreted in the light of the clinical findings. A positive L.E. test alone does not constitute an absolute criterion for the diagnosis, nor does it exclude the disease when the test is negative.

The present observations indicate that the reaction occurs most characteristically in acute disseminated lupus erythematosus, being positive in the majority of patients with this disease. However, it may also be positive in a smaller proportion of other diseases. Whether there is any relationship between these various disorders on the basis of a common abnormality of the immune mechanism remains to be determined.

The phenomenon emphasizes the close relationship which exists between disseminated lupus erythematosus on the one hand and rheumatoid arthritis on the other. There is little doubt that some patients with rheumatoid arthritis may exhibit L.E. cells. In the present survey 17 such patients have been observed with positive tests. All had the classical picture of chronic rheumatoid arthritis, present in some instances for periods as long as 31 years and associated with typical rheumatoid nodules. Some have shown evidence of systemic and visceral involvement,[82] while others exhibited only the characteristic joint deformities and juxta-articular nodules, without evidence of visceral disease. Several had been subjected to arthroplasty or other surgical procedures for correction of deformities. In two of the patients the test was initially negative when they were seriously disabled and had a leukopenia (1500-3000 cells per c.mm.), but later became positive when the white cell count was appreciably increased after prednisone therapy and at a time when they were greatly improved symptomatically.

The relationship of the two diseases is further emphasized by the observations that sensitivity to hydralazine may sometimes induce the typical syndrome of systemic lupus erythematosus with L.E. cells and at other times simply the manifestations of rheumatoid arthritis. Both have been reversible when the drug has been withdrawn. The frequent overlapping of the manifestations of the two diseases raises the question whether they constitute separate and distinct clinical entities, or related disease syndromes dependent upon multiple etiological factors.

Until the nature of the phenomenon is better understood, one must be cautious of the pitfalls of labelling as lupus erythematosus all diseases in which L.E. cells may be demonstrated. Much has been written but more investigation is necessary before the full significance of the reaction

is known. Undoubtedly the test has made the diagnosis of lupus erythematosus easier in many cases formerly in doubt, but there is risk of greater confusion if this broad spectrum of diseases is grouped under one diagnosis. The occurrence of the reaction in different diseases does not imply that identically the same factor is responsible in all cases, although the mechanism of production of the characteristic cells may be the same. For the present it would seem better to relate the phenomenon to a reactive state of the tissues associated with an abnormality of the immune mechanism which may occur in a variety of morbid states.

SUMMARY

The most significant feature of the L.E. (lupus erythematosus) cell reaction as it occurs *in vitro* is an unusual type of degeneration which affects some of the white cells of the blood or bone marrow, in the presence of the L.E. factor, with lysis and ultimate release of their altered nuclei. Some of the amorphous nuclear masses thus released are ingested by actively phagocytic leukocytes to form L.E. cells. Virtually all the white cell elements of the peripheral blood may take part in the phenomenon. The phagocytic stage of the reaction and the formation of the characteristic rosettes are of lesser significance and appear to represent a physiological response in the exclusion of foreign material from the blood.

While the L.E. phenomenon occurs most characteristically in acute disseminated lupus erythematosus, it cannot always be demonstrated in this disease, and may occasionally be positive in other disorders. In the present study it was found to be positive in 17 patients with rheumatoid arthritis, three patients with chronic hepatitis, and one patient each with scleroderma, dermatomyositis, polyarteritis nodosa, acquired hæmolytic anæmia and Hodgkin's disease. It was also positive in two patients who suffered reactions to phenylbutazone and in four patients following a reaction to prolonged hydralazine therapy. These observations suggest that the test has a more general significance in relation to the antibody-producing mechanism, is not entirely specific for disseminated lupus erythematosus and must be interpreted in the light of the clinical findings.

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RÉSUMÉ

Le trait le plus caractéristique de la réaction cellulaire du L. E. (lupus érythémateux disséminé aigu exanthématique) produite in vitro est une sorte de dégénérescence bizarre qui affecte certains globules blancs du sang ou de la mœlle, en présence du facteur L.E., avec lyse des cellules, ainsi que libération et modification de leurs noyaux. Une partie des masses nucléaires amorphes ainsi libérées est absorbée par des leucocytes phagocytaires pour devenir des cellules L.E. Virtuellement, tous les éléments des globules blancs du sang peuvent prendre part au phénoméne. Le stage phagocytaire de la réaction et la formation des rosettes caractéristiques importent moins et semblent représenter une réponse physiologique à l'élimination d'éléments étrangers au sang.

Bien que le phénomène L.E. se produise de la manière la plus caractéristique dans le lupus érythemateux disséminé aigu, il n'est pas toujours possible de le démontrer dans cette maladie. Il peut par contre être positif quelquefois dans d'autres maladies. Dans la présente étude, l'auteur obtint des résultats positifs chez dix-sept malades souffrant d'arthrite rhumatoïde, chez trois autres atteints d'hépatite chronique, et dans un cas de chacune des maladies suivantes: la sclérodermie, la dermatomyosite, la polyartérite noueuse, l'anémie hémolytique acquise, et la maladie de Hodgkin. Le phénomène fut aussi positif chez deux malades qui souffraient de réactions au phénylbutazone, et chez quatre autres à la suite d'un traitement prolongé à l'hydralazine.

Ces observations laissent supposer que cette épreuve a une portée plus générale en relation avec le mécanisme de production des anticorps, qu'il n'est pas absolument spécifique au lupus érythémateux disséminé, et qu'il doit être interprété à la lumière des constatations cliniques. M.R.D.

THE RELATION OF EMOTIONAL FACTORS TO RECURRENCE OF THYROTOXICOSIS*

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THE CLINICAL STUDY which forms the basis of this article was undertaken at the suggestion of local endocrinologists who were dissatisfied with the long-range results of medical and surgical treatment of their thyrotoxic patients, especially as far as recurrences of the disease are concerned. The aim of the study was to ascertain whether cognizance of emotional factors might be of help in understanding the mechanism of recurrences and might contribute to more predictable management of thyrotoxic patients.

In particular, we set out to determine how the course of the illness and the patient's response to medical help might be related to: (1) The way in which the illness fits into the patient's life pattern. (2) The way in which the patient interprets his symptoms. (3) The way in which the patient interprets his treatment. (4) The way in which psychophysiological healing occurs.

REVIEW OF THE LITERATURE

Ever since the original observations by Parry and by Graves over 100 years ago of the connection between emotional experiences and the onset of thyrotoxicosis, the relevance of emo-

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