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L.E. FACTOR TEST
IN ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

BY
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A THESIS

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INTRODUCTION

Systemic lupus erythematosus is a rare disease. And like other rare diseases it becomes scarcer through lack of consideration. Therefore a high index of suspicion is necessary if the diagnosis is to be made more frequently. Before the discovery of a new diagnostic test, acute disseminated lupus erythematosus was a medical curiosity. A few years ago the diagnosis was made with certainty only when the classical picture was encountered. A few astute physicians made the diagnosis in obscure cases but always with some hesitancy. A typical cell was discovered which is diagnostic of the disease and an entity once obscure assumed prominence in medical literature. The profession became more conscious of the disease because with a laboratory procedure there was more confidence in the diagnosis. The realization that the diagnosis has frequently been missed is becoming apparent with the utilization of diagnostic laboratory procedures.

In January 1948, Hargraves, Richmond and Morton (1) announced the discovery of a new cell in bone marrow preparations of patients with acute disseminated lupus erythematosus. Hargraves gave the name L.E. cell to this new entity. A chain of investigations has evolved a new diagnostic procedure (2, 3, 4) for this disease. Lupus erythematosus is now prominent in medical literature and is the subject of a great deal of study. Reports concerning
the specificity of this cell for systemic lupus erythematosus are appearing in the literature. (7, 8, 11)
L.E. CELL

The L.E. cell is usually a mature polymorphonuclear leukocyte in which there is a homogeneous, purple staining mass in the Hof of the nucleus. Hargraves et al. (1) in their description of this cell discussed a similar cell, the tart cell, from which the L.E. cell must be differentiated. The tart cell is a monocytic histiocytic with a secondary nucleus in the Hof. This secondary nucleus has a distinct nuclear membrane and sharply differentiated chromatin. It is found in normal marrows in small numbers, however, there are certain diseases in which they are seen with greater frequency. These are lymphoblastoma, pulmonary infections, and metastatic carcinomas.

The L.E. cell on the other hand is a mature polymorphonuclear leukocyte with a homogeneous purple staining mass in the Hof. And, although, a few of these cells may show chromatin pattern, thereby simulating tart cells, most of the L.E. cells on the slide have no chromatin pattern.

Questions have naturally arisen concerning the origin of this engulfed purple staining, homogenized inclusion body. There is no unanimity of opinion on the nature of this material. Haserick et al. (5) believe it to be either of platelet or megakaryocytic origin, or an amorphous protein. Hargraves and his group (1) suggests a phagocytosis of free nuclear material resulting in a round vacuole containing this partially digested
and lysed nuclear material, or, an actual autolysis of one or more lobes of the nucleus.

While Hargrave (1) was able to demonstrate the L.E. cell many investigators failed to duplicate his results. Hargraves (1) pointed out that failure was because the direct smear method of preparing bone marrows was being utilized by these investigators. In order to obtain L.E. cells the concentration method is necessary. This procedure allows the plasma to contact cells. This is necessary for the formation of L.E. cells. Hargraves (2) pointed out that L.E. cells could be found in peripheral blood, also, but only if a concentration method is used, similar to that used with bone marrow preparations.

The possibility of this phenomenon being due to the anticoagulants used occurred to Hargraves. (2) He produced the L.E. cells using various anticoagulants thereby exonerating these preparations as a cause of the formation of the cell. Others have demonstrated the cells in clotted blood in which no anticoagulant was used. (11)
Further search of the L.E. phenomenon was undertaken by Haserick and Bortz. (3, 4) The importance of the plasma in forming the L.E. cell has already been mentioned. These investigators studied the effects of mixing plasma from patients with acute disseminated with normal bone marrow. One-half cubic centimeter of plasma was added to one cubic centimeter of heparinized bone marrow. This was mixed and allowed to stand for one-half hour. The resultant mixture was concentrated according to Limarzi’s technique by placing it in a hematocrit tube and centrifuged at 1000 R.P.M. for five minutes. The myeloid-erythroid layer was taken off with a small amount of plasma and smears were made and stained with Wright's stain. Three tubes were used. The first tube contained normal bone marrow with nothing added. The second tube consisted of normal bone marrow plus plasma of a normal patient. The third tube had normal bone marrow with plasma from a case of disseminated lupus erythematosus.

Changes occurred only in the third tube. These consisted of two types. The first was a chemotactic attraction of polymorphonuclear leukocytes around a purple homogenous mass. These were called rosettes. The number of polymorphonuclear cells in each rosette varied from two to ten. The second observation was the formation of typical L.E. cells.
Serial smears of L.E. plasma and bone marrow mixture reveal that rosettes of clumped leukocytes are seen at 5 to 6 minutes. L.E. cells, if they appear at all, are seen at 12 to 15 minutes. The conclusion then, is that L.E. cells are the end result of the phenomenon of leukocyte clumping. A positive test need not have typical L.E. cells. The clumping phenomenon in itself is diagnostic.

Haserlck and Bortz (3, 4) concluded from these experiments that there was an element in the plasma that caused the L.E. phenomenon. Hargraves (2) also reported these results. This was the introduction to the L.E. factor test.

Continuing his work Haserlck and Bortz (4) using the same technique compared plasma of suspected cases of acute lupus erythematosus with known cases. They again used three tubes. In the first tube there was normal bone marrow only. The second tube contained normal bone marrow mixed with plasma from a suspected case of acute disseminated L.E. The third tube consisted of normal bone marrow and plasma of proven cases of acute disseminated L.E. Comparisons were made after concentration and preparation of smears. The rosettes of polymorphonuclear cells, and L.E. cells were produced only in cases of acute disseminated L.E. Plasma from fourteen patients with acute disseminated L.E. gave the typical phenomenon. It was induced in twenty-seven normal bone marrows. Later this group reported the plasma of one patient
with acute disseminated L.E. inducing a positive L.E. plasma test in forty-six consecutive bone marrow preparations, another in forty-three and another in thirty-six.

The L.E. factor test as it evolved from this chain of investigation has become an important diagnostic tool. To date with a few isolated exceptions (10, 11) it is a specific test for acute disseminated L.E. The plasma L.E. test has been found to be negative in many diseases such as chronic discoid L.E., rheumatoid arthritis, multiple myeloma, scleroderma, periarteritis nodosa, cirrhosis of the liver, rheumatic fever, dermatomyositis and other miscellaneous disorders. (3, 4) These conditions were selected for study because some are collagen diseases as in acute disseminated lupus erythematosus or are associated with hyperglobinemia. Of course, a great deal more investigation will be necessary in order to definitely prove that this test is specific. While L.E. cells occur with much greater frequency in systemic lupus erythematosus an occasional occurrence with other diseases has been reported. (8, 10, 11) The diseases in which L.E. cells were reported include one case of multiple myeloma, one pernicious anemia relapse, one leukemia and one amyloidosis. (8)

A patient with acute disseminated lupus erythematosus may be a seriously ill patient or may have a marked leukopenia with a hypoplastic bone marrow, which is characteristic of the
disease. These situations make a bone marrow study technically difficult. But with a simpler procedure available a study of the patients bone marrow is not necessary. The L.E. factor test requires only a small sample of blood removed by venipuncture method. Also the L.E. factor test may be positive while the patients own marrow may not show L.E. cells. This was found in three patients in Hasericks series. (4) This indicates that the L.E. factor test is a more diagnostically important test than the study of the patients marrow.

Bone marrow techniques are occasionally impractical since in private practices it is not always available. Investigators (9) have sought a method whereby peripheral blood could be utilized. A search for L.E. cells in peripheral blood of patients with acute disseminated lupus erythematosus is time consuming and often fruitless. Moffatt, Barnes and Weiss (9) solved this dilemma using a mixture of peripheral blood from a normal patient with blood from the suspected case. The procedure consisted of 0.5 c.c. heparinized blood from suspected case mixed with an equal amount of heparinized venous blood from a normal patient. This preparation is allowed to stand twenty minutes and is then centrifuged for five minutes at 2000 R.P.M. The Buffy coat is removed with a pipette and smears are made. These smears are stained with Wright's stain. The typical L.E. cells are demonstrated by this simpler method as well as the rosettes of polymorphonuclear
leukocytes. This method is simpler and is one that is accessible to all practitioners. It obviates technical difficulties such as inability to obtain marrow and requires no special techniques.

Another method (7) of using peripheral blood instead of bone marrow is adding of 0.5 c.c. plasma to 1 c.c. normal peripheral blood and following the concentration technique as before. This test is then performed in exactly the same way as the original L.E. factor test with the exception that peripheral blood is substituted for bone marrow.

Dog marrow has been used by Haserick (7) to perform the L.E. test. But only clumping of leukocytes occurs. There are no typical L.E. cells produced.

Further examinations on the L.E. factor revealed a number of interesting observations. Of a practical nature was the investigation of Haserick of bacterial action on the L.E. factor. (3) He pointed out that bacterial invasion of the plasma destroyed the L.E. factor. To overcome this objection, samples of plasma to be used for testing purposes must be taken under sterile conditions. Samples can be refrigerated to prevent bacterial growth and if the test is not to be done immediately the sample should be kept in a refrigerator. If suspected plasma is to mailed long distances powdered penicillin can be added to prevent bacterial growth. Merthiolate or benzyl alcohol should not be added because,
like bacteria, they produce a false negative test.

Heating plasma to 65°C L.E. cells do not appear and at 65°C clumping of leukocytes fails to occur. L.E. factor is stable at room temperature for six months. Our experience on plasma kept under sterile conditions in refrigerated compartments with routine care has been illuminating. We find that the plasma looses its potency in about two weeks and that fewer L.E. cells are formed as time increases. Clumping of leukocytes is less prominent during the third week. This, of course, is with routine care and ordinary sterile precautions as would be used in any hospital. Because of this observation for practical purposes it is recommended that the test be done as soon as possible and preferrably within a week of obtaining the specimen of plasma.

The effect of drugs on the L.E. factor is interesting as well as important. There have been a few reports that ACTH and cortisone has been useful in treating acute disseminated L.E. and in some instances have caused a disappearance of L.E. cells from the patients marrow. Our experience in performing the L.E. factor test after cortisone and ACTH therapy has shown no difference before and after treatment. Other drugs that do not inhibit the L.E. factor when added to L.E. plasma are penicillin, nitrogen mustard, estradiol, progesterone, chronic discoid lupus erythematosus plasma, and testosterone. Further investigation along these lines is indicated. It seems plausible that if a substance can be found that will inhibit the L.E. factor it will perhaps prove to be efficacious in treating the disease.
SPECIFIC PLASMA FRACTION

When plasma was found to be responsible for inducing the L.E. phenomenon many problems appeared. What portion of the plasma is concerned with producing the clumping of leucocytes and the formation of L.E. cells? This question became foremost in the minds of investigators. Haserick, Lewis and Bortz (5) undertook the task to answer the question. Fractions of plasma samples were mixed with normal bone marrow to determine which portion induced the phenomenon. Fractionation was done on the Tisclius apparatus. The fractions used were: 1) albumin (3.5 mgm), 2) albumin, alpha and beta globulin (12 mgm), 3) gamma globulin (2 mgm), 4) gamma globulin and fibrinogen (4.8 mgm). Each fraction was added to normal bone marrow and the L.E. test was performed on each. The fractions producing leukocyte clumping and L.E. cells were the gamma globulin, gamma globulin and fibrinogen, and whole plasma. It was noted that whole plasma gave a more clear cut action than any fraction.

It is therefore clear from this work that the L.E. factor is in the gamma globulin portion of the plasma. Although this factor has been identified within the gamma globulin fraction of L.E. plasma, the exact connection has not been determined.
ANTIBODIES AGAINST L.E. FACTOR

Using the gamma globulin fraction from positive L.E. plasma antibodies were produced in rabbits. (6) This was accomplished by the injection of gamma globulin fractions of L.E. plasma into rabbits three times weekly. Anti L.E. gamma globulin antibodies were thereby induced in the rabbits. In order to test inhibition of L.E. factors by antibodies developed in rabbits, L.E. gamma globulin was added to the anti L.E. gamma globulin rabbit serum. This was allowed to stand and the precipitate was removed. The supernatant fluid was added to normal bone marrow and the L.E. factor test was done. Neither clumping of leukocytes nor L.E. cells were observed if the precipitin titer of the anti L.E. gamma globulin rabbit serum against the L.E. gamma globulin was 1:80 or greater. This indicated complete inhibition of the L.E. factor by the anti L.E. gamma globulin rabbit serum. The addition of the anti L.E. gamma globulin to L.E. gamma globulin caused precipitation of the L.E. factor.

The same procedure was used again but the L.E. gamma globulin was added to the serum of rabbits immunised with normal serum. The supernatant fluid, after precipitation induced the L.E. phenomenon. This definitely showed that the L.E. factor was not precipitated.
The purpose of this study was to determine a possible immunologic difference between L.E. gamma globulin and normal gamma globulin. The difference was thought to be the L.E. factor as the above investigation indicates. The study showed that L.E. gamma globulin consists of: normal gamma globulin which is precipitated by the anti sera against the normal human serum, and the L.E. factor which is not precipitated by these antibodies. These studies suggest that the L.E: factor is an immunologically distinct component of the L.E. gamma globulin because of the immunologic difference between the L.E. factor and gamma globulin.
PROBLEM AND METHOD

Recent investigation on the L.E. factor test has been to determine its specificity (7, 10, 11) for acute disseminated lupus erythematosus. The investigations in other diseases has been previously cited. A great deal of work must be done as yet in searching for this phenomenon in other diseases. This problem was the subject of this study. Cases were selected for reasons which will be explained. Some particular disease groups have already been investigated but the nature of the disease justifies further search to add to the small number done in these groups. Reference to the collagen group of diseases in particularly made since acute disseminated lupus erythematosus belongs to this group. Only a small number of cases have been checked with the L.E. factor test. A great many tests will be required before it can be said with certainty that the test is specific for only one disease entity, namely acute disseminated lupus erythematosus. Results have been encouraging indicating that it will be diagnostic of this particular disease.

Haserick's procedure (4) was used as the pattern in this investigation. One-half cubic centimeter of plasma from the patient to be investigated was added to one cubic centimeter of normal bone marrow. The resultant mixture was allowed to stand for one-half hour at room temperature. Using Limarzi's
concentration technique the mixture was prepared for smears. This was accomplished by placing the mixture of plasma and bone marrow in a Wintrobe hematocrit tube and centrifuging the specimen for ten minutes. The myeloid-erythroid or buffy layer was removed with a pipette and transferred to a watch crystal with a small amount of the plasma layer. This was carefully mixed and smears were made. Wright's stain was used. The slides were stained Wright's stain for two minutes. Buffer was then added for eighteen to twenty minutes. The staining procedure was done on one slide initially, the staining time being varied according to the outcome.

All the bone marrows used came from hematologically normal patients in our clinic wards. These marrows were checked using the same concentration procedure to verify their normalcy.

In all positive cases more than one marrow was used. In the first positive case the plasma was checked on six different bone marrows. In the second positive case the plasma was tried on three different marrows. In the third and fourth, two marrows apiece were used to check the plasma from each cases. In all tests positive results occurred.
RESULTS

Of the collagen group of diseases the test was positive only in acute disseminated lupus erythematosus. These four cases will be described more fully in the case reports. The plasma from three cases of acute rheumatic fever gave negative tests. Plasma from four cases of rheumatoid arthritis was negative. Three cases were in the early acute stages while a fourth patient had been afflicted with the disease for three years. One case of periarteritis nodosum gave a negative L.E. plasma test.

For obvious reasons chronic lupus erythematosus was checked. Five cases of this disease gave consistently negative results. This adds support to the tenum that the chronic discoid variety is not a systemic disease. We had no opportunity to examine the results in cases of acute exacerbations, in this disorder.

A patient with multiple myeloma with a hyperglobulinemia gave a negative test indicating that even though the plasma globulin is increased in this disease, it does not contain the factor responsible for the L.E. phenomenon.

Other cases in which the L.E. factor test was negative were four skin disorders and one case of nephritis. The skin cases considered were acute erysipelas, chronic eczematoid dermatitis, and two cases of contact dermatitis.
TABLE 1

L.E. FACTOR TEST

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>Diagnosis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Acute Rheumatic Fever</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Rheumatoid Arthritis</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>Periarteritis Nodosum</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Chronic L.E.</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>Nephritis</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>Multiple Myeloma</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Skin Disease</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Acute L.E.</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Four cases of acute disseminated lupus erythematosus gave positive results. A positive test consisted in the demonstration of typical L.E. cells and clumping of polymorphonuclear leukocytes in rosettes.
Fig. 1. Typical L.E. cell, a polymorphonuclear leukocyte in which there is an homogenous purple staining mass in the Hof.
Fig. 2. Rosette composed of two polymorphonuclear leukocytes around an amorphous purple mass.
Fig. 3. Rosettes of clumped polymorphonuclear leukocytes around an amorphous purple mass.
Fig. 4. Clumping of polymorphonuclear leukocytes around purple staining mass.
The typical L.E. cells were polymorphonuclear cells in which there was a purple staining homogenous mass in the Hof. The rosettes were composed of clumping of leukocytes around an amorphous purple mass. The greatest accumulation of L.E. cells and rosettes of polymorphonuclear leukocytes was found at the ends of the slide and along the edges. All four cases produced positive tests in more than one normal marrow. The plasma from the first case induced a positive L.E. test in six different bone marrows. The second case yielded positive results in three different bone marrows. The third and fourth cases gave positive findings in two different bone marrows.

An interesting observation was that one of the patients had been on cortisone therapy for over a year and the tests were positive.

It was noted in all cases that the potency of the plasma was reduced when stored over three weeks. At first the L.E. cells disappeared and in the fourth week, even the rosettes of leukocytes became scarce and finally disappeared. The plasma during this time had been stored in a refrigerator. From this observation it was concluded that the test should be done as soon as possible after the plasma is obtained preferably within the first week and definitely before the third week.
The four cases that gave positive results when checked with the L.E. factor test deserve further scrutiny since they illustrate several important points concerning this disease.

Case 1. Mrs. W.B. aged 26 years, entered the hospital December 22, 1950 with the chief complaint of fever "off and on".

On September 21, 1950 the patient experienced a moderately severe low back pain which radiated down the left hip, few days later nausea and vomiting occurred. Diarrhea appeared along with a temperature of 104° C. She saw her doctor and laboratory findings showed a white blood count of 2600. The differential count was normal.

The fever continued intermittently and on October 22 an agglutination test for Brucellosis was reported as negative. A heterophil antibody test was positive with a titer of 1:1792. The doctor assumed that he was dealing with a case of infectious mononucleosis at this time.

On December 4 there was an exacerbation of symptoms associated with occasional joint pains in the fingers. There was no spleen and no lymph nodes.

On December 20 a heterophil antibody was positive with a titer of 1:14. Because the symptoms were persistent and progressively getting worse the patient was sent to the hospital.

The physical examination was negative with the exception
of an intermittent fever ranging up to 104° at times but usually up to 100° or 101° every few days.

An x-ray of the chest was negative. An x-ray of the lumbosacral spine revealed an anomalous development in the lumbosacral region with a transition type vertebra that is sacralized on the left.

Laboratory examination revealed a negative urinalysis on three occasions with the exception of a slight trace of albumin. The remainder of the laboratory work is listed under Table 2.

**TABLE 2**
LABORATORY DATA

<table>
<thead>
<tr>
<th>1. Blood Counts</th>
<th></th>
<th>2. Sedimentation rate - 36 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-23-50</td>
<td></td>
<td>3. Blood culture - no growth</td>
</tr>
<tr>
<td>R.B.C. 3,350,000</td>
<td>R.B.C. 3,650,000</td>
<td>4. Heterophile antibody-negative</td>
</tr>
<tr>
<td>Hb 67%</td>
<td>Hb 74%</td>
<td>5. Bence Jones proteinuria - negative</td>
</tr>
<tr>
<td>W.B.C. 3800</td>
<td>W.B.C. 3900</td>
<td>6. Total proteins - 6.0 gms. %</td>
</tr>
<tr>
<td>Segs 54</td>
<td>Segs 64</td>
<td>albumin - 2.7 gms. %</td>
</tr>
<tr>
<td>Bands 9</td>
<td>Bands 6</td>
<td>globulin - 3.3 gms. %</td>
</tr>
<tr>
<td>Lymph 37</td>
<td>Lymph 30</td>
<td>7. Bone Marrow studies showed typical L.E. cells.</td>
</tr>
<tr>
<td>12-30-50</td>
<td></td>
<td>8. L.E. factor test - positive on six successive normal bone marrows.</td>
</tr>
</tbody>
</table>

| 6. Total proteins - 6.0 gms. % |
| albumin - 2.7 gms. % |
| globulin - 3.3 gms. % |
| 7. Bone Marrow studies showed typical L.E. cells. |
| 8. L.E. factor test - positive on six successive normal bone marrows. |
Progress: The patient has been on salicylates, ACTH, and general supportive therapy. No definite improvement has been noted except the normal remission that occur with the disease. When relapses occur the patient is hospitalized for further treatment.

Comment: This represents a case of acute disseminated lupus erythematosus without skin manifestations and with no urinary findings thought to be infectious mononucleosis or a malignant lymphoma. Bone marrow studies revealed the typical L.E* cells. The L.E. factor test was consistently positive.

Case 2: Miss C.T., aged 16 years, entered the hospital January 20, 1951 with the chief complaint of intermittent joint pains of six weeks duration following an upper respiratory infection. Initially, there was transient swelling and redness of the left knee. The right knee then became involved and subsequently the ankles. After subsiding almost entirely the pains and swelling recurred in the wrist and metacarpalphalangeal joint. She complained of bouts of chills and fever especially at night.

Physical examination at the time of entry to the hospital showed a tachycardia of approximately 100 beats per minute. There were no murmurs. The examination was otherwise negative.

While in the hospital petechiae appeared under the fingernails and the spleen became palpable and markedly tender. The
spleen remained palpable for five days. Erythema nodosum occurred on palms of hands, lasting for a few days.

TABLE 3
LABORATORY DATA

1. Blood counts

<table>
<thead>
<tr>
<th>Date</th>
<th>Hb.</th>
<th>R.B.C.</th>
<th>W.B.C.</th>
<th>Segs</th>
<th>Lymph</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-21-51</td>
<td>53%</td>
<td>3,450,000</td>
<td>6,400</td>
<td>65%</td>
<td>35%</td>
</tr>
<tr>
<td>1-27-51</td>
<td>62%</td>
<td>3,430,000</td>
<td>4,100</td>
<td>61%</td>
<td>26%</td>
</tr>
</tbody>
</table>

2. Urinalysis - negative (3 occasions)

3. Sedimentation rate - negative (3 times)

4. Blood cultures - negative

5. Brucella agglutination tests - no agglutination

6. Total proteins - 4.8 gms. %
   albumin - 3.3 gms. %
   globulin - 1.5 gms. %

7. Electrocardiograms - normal

8. L.E. factor test - positive

A tentative diagnosis of acute rheumatic carditis with subacute bacterial endocarditis was made. A full course of penicillin was given. Cortisone had been started for treatment of the rheumatic carditis.

On 2-14-51 the L.E. factor test was done and was positive.
It was repeated on two more normal marrows with positive results.

The patient was discharged on 1-3-51 still on cortisone therapy. A few days after dismissal from the hospital, the patient developed swelling of the face, a hypertension and a full blown nephritic picture with many red blood cells in her urine.

She was sent to another institution and the remainder of our follow up is taken from their report. She entered the hospital acutely disturbed and delirious. Edema of the glottis necessitated a tracheotomy. There was marked edema of the face and neck. Papilloedema was present on funduscopic examination.

Sternal marrow studies revealed many L.E. cells. Laboratory examinations showed a normal white count and differential count. The hemoglobin was 7.9 gms and the red cell count 2.35 million. The urine contained grade 3 albuminuria and many red cells. The sedimentation rate was 134 mm. per hour by the Westergren method. The blood urea was 58. Electrolyte determinations of CO₂, chlorides, potassium and sodium were within normal limits.

The patient expired on March 1, 1951 and an autopsy was performed. It revealed a marked ascites with 2500 c.c. of fluid within the peritoneal cavity, 2000 c.c. in each pleural cavity and 250 c.c. in the pericardial sac. The kidney showed findings compatible with lupus erythematosus. The heart revealed an acute non-bacterial verrucous endocarditis (Libman Sachs).
Comment: This represents a case of acute disseminated lupus erythematosus that simulated bacterial endocarditis with rheumatic carditis. A full blown classical clinical picture of acute disseminated lupus erythematosus developed despite cortisone therapy. The L.E. factor test was positive while the patient was on therapy. Autopsy confirmed the diagnosis. Cutaneous manifestations were absent at the time the diagnosis was made.

Case 3: Mrs. A., aged 27 years, complained of fatigue and painful swelling of the interphalangeal joints of both hands shortly after her marriage a few years ago. Subsequently the metacarpophalangeal joints, wrists, elbows, shoulders, jaws, knees, ankles, toes and heels became involved. Motion of these joints produced pain. Fever up to 101°F daily accompanied the joint symptoms. Anorexia, nausea and weight loss were present. She was treated with gold, pregnenolone, glucuronic acid, transfusions, aureomycin and vitamins without much relief. She had typical migraine headaches. Tightness in chest associated with a brassy dry cough and some dyspnea was present.

Physical examination on September 5, 1950 revealed a herpetiform type skin lesions on buttocks which had been present for seven years. There was a nodular non-toxic thyroid. The shoulders were painful on extreme motion. The wrists, ankles and fingers were tender. There was generalized muscular tenderness.
The laboratory examination revealed the following pertinent facts: a white blood count of 3,650, a sedimentation rate of 115 mm/hour (Westergren method).

A provisional diagnosis of possible disseminated lupus erythematosus or rheumatoid arthritis was made and 100 mgm. Cortisone per day, intramuscularly was started on September 15, 1950. Considerable improvement was noted but evening edema, and pericardial effusion developed. These findings with their resultant symptoms were relieved by a low sodium diet.

An L.E. test done elsewhere was reported positive. Cortisone therapy was discontinued in November 1950 followed by a violent exacerbation of the systemic disorder. This was characterized by high fever, severe pain, pleural and pericardial effusions. Cortisone was started again with gradual improvement. The sedimentation rate was 88 mm per hour.

On February 14, 1951 the patient entered the Offutt Air Base hospital and improvement occurred. There was diminished swelling and pain of joints and an increase in mobility of the joints. She complained of pressure in the upper substernal region radiating into the neck and face occurring without any reason. Fever subsided.

Undesirable side effects produced by the cortisone therapy were edema, increase in hair on face, arms, body and legs cessation of menses for two months, generalized osteoporosis on x-ray and thickening of the buccal fat pads.
Physical examination at this time showed involvement of the knee joints with effusion. The remainder of the examination was similar to the previous examination.

The urinalysis and blood count were normal. The serum albumin was 4.13 grams percent and the globulin 2.78 grams percent.

Roentgenograms revealed pericardial effusion which was substantiated by fluoroscopy.

Cortisone therapy was continued. An L.E. factor test was done by us and was positive with two different bone marrows.

Comment: This case illustrates several points the most important of which is the strong positivity of the L.E. plasma test despite long continued cortisone therapy to the point of undesirable side effects.

Case 4: Miss M.G., aged 9 years, entered the hospital with the chief complaint of a rash of butterfly pattern over the face. This skin rash began ten days prior to admission beginning at the hair line. The following day it was a fiery red "blotchy" rash over the bridge of the nose and over the cheeks. The patient complained of easy fatiguability.

Physical examination showed an erythematous rash over the bridge of the nose and over the cheeks. It extended over the forehead to one centimeter below the hairline. This was a fiery red blush with no scaling and no elevation. The physical examination was otherwise negative. The child was of normal
development for her age and did not appear to be precocious.

An x-ray of the chest revealed increased hilar markings.

Routine laboratory examinations were not unusual. The urinalysis was negative with the exception of 3 to 4 red blood cells per high power field. The blood count was within normal limits. W.B.C. 5,350 with a normal distribution. A throat culture showed streptococcus viridans, sensitive to aureomycin and chloromycetin.

A thorn test was not too revealing. An L.E. factor test was done and was positive. The typical L.E. cells were seen as well as rosettes of clumped leucocytes. The test was positive on two normal bone marrows.

Comment: This case is interesting primarily because of the age of the patient. It is unusual to find disseminated lupus erythematosus in females before the age of puberty. The possibility of precocious development of the child must be considered but there was no evidence to confirm it. The paucity of systemic findings warrants further observation of this patient. With the typical rash of disseminated lupus erythemasus and the positive L.E. factor test this case must be considered as such until proven otherwise.
SUMMARY AND CONCLUSIONS

Over the span of a few years interest has been renewed in acute disseminated lupus erythematosus largely because of the discovery of the L.E. cell by Hargraves. (1) The necessity of utilizing concentration methods of preparing bone marrow in order to demonstrate these cells prompted investigation which resulted in incriminating the plasma fraction of the blood as the agent responsible for the formation of L.E. cells. The plasma portion responsible for the L.E. phenomenon is contained in the gamma globulin fraction. (5) From this observation evolved the plasma L.E. test (2, 3, 4) which has previously been described. The finding of L.E. cells and/or rosettes of clumped polymorphonuclear leukocytes constitutes a positive test.

It was the purpose of our investigation to study the specificity of the L.E. factor test in disseminated lupus erythematosus. A number of cases from the collagen group of diseases were checked as shown in Table 1. Of this group only cases of disseminated lupus erythematosus gave positive tests. Chronic lupus erythematosus gave consistently negative results in five cases. Multiple myeloma, nephritis, erysipelas, contact dermatitis and chronic eczematoid dermatitis were negative. A total of nineteen cases gave negative results to the test.

Four cases of disseminated lupus erythematosus gave
positive tests repeatedly. Only when the plasma from these cases was stored over three weeks did negative slides result. It is therefore advised that the test be done as soon as feasible after the plasma is obtained. In studying the slides although the cells are well distributed we found the greatest accumulation of L.E. cells and rosettes of clumped leukocytes at the ends of the slide and along the edges, that is, the peripheral portion of the smear. Cortisone therapy did not affect the results of the test.

Four cases of disseminated lupus erythematosus are presented to show the diverse clinical pictures. In only one case was the classical butterfly pattern of rash present. All cases were females. The last case was unusual because of its occurrence in a nine year old girl.

The L.E. factor test is a simple test although time consuming. It should be utilized more often in the future. It is a test requiring a minimum of equipment and can be used in the doctor's office as well as in the hospital. If physicians will take advantage of the test a great many more cases of systemic lupus erythematosus will be discovered and in the future we shall find that it is not such an extraordinary disease after all and that the diagnosis has frequently been missed. It is recommended that the test be done on all patients with rheumatoid arthritis and any one of the following: epilepsy, syphilis (false positive
tests), albuminuria, leukopenia. It should also be routinely
done in all undiagnosed fevers and in patients suspected of acute
rheumatic fever, especially female patients past the age of
puberty.
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