SIMPLE DETERMINATION OF THE L.E. FACTOR

LOOSE BODY TEST AND NUCLEUS AGGLUTINATION INHIBITION TEST

BY

F. VAN SOEREN

*Laboratory for General Pathology, University of Amsterdam
(Director: Prof. P. Formijne, M.D.)*

The diagnosis of systemic lupus erythematosus is based on the four following points:

(1) *The Clinical Picture*.—This does not always present itself in the complete classic form, as described by Osler (1895). The incomplete picture is difficult to recognize, and may appear as lupus arthritis, lupus nephritis, lupus hepatitis, and lupus thrombocytopenia.

(2) *The L.E. Cell Test*.—In about 80 per cent. of the proved cases the Hargraves test is positive. A theoretical objection to this test is that it employs phagocytosis which is a secondary reaction *in vitro* as indicator. The fixation of the specific L.E. gamma globulin to nuclei, which constitutes the first phase of the L.E. cell formation and results in loose body formation (Aisenberg, 1959; Rifkind and Godman, 1957; Rohn and Bond, 1952; Robineaux, Buffé, and Kourilsky, 1956), is not considered sufficient proof, but it is accepted that in some cases phagocytosis cannot occur because S.L.E. serum inhibits phagocytosis (Finch and Detre, 1958), has a lowered complement content in the active phase, and may show anti-complementary activity (Formijne and van Soeren, 1958).

The practical disadvantages of the L.E. cell test are its laboriousness and the reading-off difficulties in weakly-positive cases.

(3) *S.L.E. Serology*.—Some of the disadvantages of the L.E. cell test have been discounted by serological methods using the reaction between the nuclear components and the L.E. factor. The attention is directed to secondary indicators such as complement fixation, antiglobulin fixation, and nucleoprotein-latex precipitation. Only the anti-globulin fixation methods have proved to be sensitive enough (Miescher, 1955; Friou, 1958a, b, c; van Loghem, van der Hart, Hijmans, and Schuit, 1958; Engelfriet, van der Hart, and van Loghem, 1959). The antiglobulin-fixation test of Friou offers the best possibilities, as it is also suitable for quantitative use.

The intricate techniques required for the serological methods are a handicap for general use.

(4) *The Pathological-anatomical Picture*.—The histological picture of S.L.E. is typical and is characterized by haematoxylin staining bodies, fibrinoid necrosis, wire-loop lesions in the renal glomeruli, and onion-skin structures in the splenic arterioles, that may be demonstrated by biopsy. The most reliable criterion, the presence of haematoxylin staining bodies (Pollack, 1959; Worthington, Baggenstoss, and Hargraves, 1959), may, however, be overlooked if one omits to study the histological sections with a higher magnification (600-800×).

In practice the diagnosis of S.L.E. is sometimes not established for several years, or comes as a surprise finding at autopsy.

There is therefore still a need for a reliable simple quantitative method of determination that meets these difficulties. We believe to have found this in the two combined methods described below:

A. *Loose Body Test (L.B.T.)*.—This microscopic method is based on the direct reaction between L.E.-factor and free leucocyte nuclei and is suitable for quantitative use (van Soeren, 1960).

B. *Nucleus-Agglutination Inhibition Test (N.A.I.T.)*.—This test is based on the fact that free leucocyte nuclei suspended in "normal" serum, form a nucleoprotein clot. The reaction does not occur if all free leucocyte nuclei are loaded with L.E.-factor. It is a macroscopic test, which can also be used quantitatively (van Soeren, 1960).

Loose Body Formation

Free leucocyte nuclei incubated in a strong S.L.E. serum, all become loaded with L.E. factor in less than a minute, and are transformed into entirely homogeneous loose bodies which are histochemically and morphologically identical to the haematoxylin staining bodies. They remain present in the serum in a homogeneous suspension and, after sedimen-

tation, can be smeared out as a homogeneous film on an object glass. Hundreds of them are seen per visual field with a low magnification (80 $\times$).

This reaction is remarkably little thermosensitive and runs its course within a minute at both 0° and 56° C.

On stepwise dilution of the L.E. serum with normal serum (1:2, 1:4, 1:8, 1:16, etc.), and incubation of such a dilution series with leucocyte nuclei suspension, entirely homogeneous and structureless loose bodies are found in the smears of the first dilutions. However, the loose bodies become gradually less homogeneous in the higher dilutions, and begin to assume a grid structure. We called this type the "rough type of loose body". These loose bodies can also be smeared out on an object glass, as a homogeneous film.

L.E. Serum	1:1	1:2	1:4	1:8	1:16	1:32	1:64
Smooth Loose Bodies	++	++	++	++	++	+	—
Rough Loose Bodies	—	—	—	+	++	++	—

Loose body formation only occurs if the test serum contains the L.E. factor. In the opposite case, a nucleoprotein clot formation is observed, and the nuclei of the leucocytes liquefy into a thready mass of nucleoprotein, no longer to be smeared out on an object glass. In a smear of normal serum incubated with leucocyte nuclei, only these liquefied nucleoprotein clots are found. These clots are also observed in the higher dilutions of an L.E. serum when not all nuclei can be loaded with L.E. factor.

The presence of these nucleoprotein clots is also macroscopically visible, and this has led us to the discovery of the macroscopic phenomenon of nucleus agglutination.

Nucleus Agglutination

Leucocyte nuclei, incubated for 10-30 minutes at 37° C. in normal serum, agglutinate by gentle shaking into a clearly visible nucleoprotein clot. This reaction is blocked at 0° C., is incomplete at 20° C., and takes its full course within 1 or 2 minutes at 56° C. If clot formation occurs on shaking, the initially opaque serum clarifies and becomes transparent. This reaction does not occur if the leucocyte nuclei have been loaded with L.E. factor beforehand. In this case there is an inhibition of the nuclear agglutination, and the suspension remains homogeneous and opaque on shaking.

The nucleus agglutination inhibition phenomenon can also be followed-up quantitatively if the S.L.E. serum is stepwise diluted with normal serum:

L.E. Serum	1:1	1:2	1:4	1:8	1:16	1:32
Nucleus Agglutination Inhibition ..	+	+	+	±	—	—

This phenomenon is directly related with the loose body formation; the rapid fixation of the L.E. factor prevents the rather slow formation of the nucleoprotein clots:

L.E. Serum	1:1	1:2	1:4	1:8	1:16	1:32
Nucleus Agglutination Inhibition ..	+	+	+	±	—	—
Loose Body Formation	++	++	++	++	+	—

In the smear preparations of dilution 1:16, the nucleoprotein clots and the rough type loose bodies are visible beside each other.

Technique of the Loose Body and Nucleus Agglutination Inhibition Tests

A typical series of results is shown in Fig. 1 (opposite).

Material and Methods

(a) *Leucocyte Nuclei Suspension.*—The leucocytes are gained from heparinized blood of patients with a high erythrocyte sedimentation rate (more than 80 mm./hr). 30 ml. heparinized blood is set aside for 30 minutes under an angle of 60°. The plasma layer that contains the leucocytes is pipetted off, centrifuged (1,500 r.p.m.), and eluted three times with a sterile buffer solution of the following composition (Aisenberg, 1959):

8 g. NaCl
1.38 g. NaH₂PO₄
1 litre distilled water
Adjusted at pH = 7.0 with NaOH

The re-sedimented leucocytes are then shaken for 5 minutes with sterile distilled water in a 5 per cent. concentration, again centrifuged (1,500 r.p.m.), and transferred to a sterile buffer solution in a concentration of 10 vol. per cent. Provided it is prepared under sterile conditions and stored at

Fig. 1.—A typical series of results.

4° C., such a nuclear suspension is tenable for some weeks.

(b) *Method of Incubation (L.B.T. and N.A.I.T.).*—20 drops of the serum under investigation are thoroughly mixed with 3 drops of nuclear suspension and incubated for 30 minutes at 37° C. in a sterile agglutination tube (8 × 80 mm.).

(c) *Quantitative Determination.*—20 drops of normal serum are pipetted into 20 drops of L.E. serum. After thorough mixing, 20 drops of the mixture are transferred to a next tube, to which again 20 drops of normal serum are added. The process is repeated after thorough mixing. Thus a series 1:1, 1:2, 1:4, 1:8, etc., is obtained. From the last tube, 20 drops are thrown away. 3 drops of nuclear suspension are added to all tubes; the incubation is done as described under (b).

(d) *Combination with the L.E. Cell Test.*—An L.E. cell test can be carried out in a simple way parallel with the test described above. For this purpose, 1 drop of nuclear suspension and 3 drops of

leucocyte suspension are added to 20 drops of serum; the incubation is likewise done for 30 minutes at 37° C.

(e) *Reading Off.*—In the first place the nucleus agglutination is read off. When the tube is carefully shaken between thumb and index finger, a macroscopically visible nucleoprotein clot is formed within about 10 seconds. This phenomenon does not arise in S.L.E. sera that contain sufficient L.E. factor.

The tubes are then centrifuged at 3,000 r.p.m. The sediment is smeared out on an object glass, dried, stained, and judged as a normal blood preparation.

Evaluation

The nucleus agglutination inhibition is only called positive if no trace of nucleoprotein clot formation is visible to the naked eye. Formation of strings on shaking, therefore, already indicates nucleus agglutination. The evaluation of the microscopical preparations is very simple.

The loose body formation is such a massive phenomenon—thousands of loose bodies are found in a single preparation—that even with a low magnification (80×) the preparation is recognized as positive.

Study with a high magnification (800×) is only necessary to judge the degree of homogenization of the loose bodies.

The negative preparations are also recognizable at once from the almost empty visual field and the appearance of nucleoprotein clots.

Between the two there is a small group of sera showing nucleus agglutination, in which a careful search of the microscopic preparation reveals some 20 to 100 rough loose bodies. These sera are recorded as sporadically positive. The same picture is found in the borderline titre of a dilution series of a positive serum. The highest dilution with massive loose body formation is, however, here accepted as the limit.

Results

Both methods were tested in 800 sera, 720 obtained at the Wilhelmina Hospital of Amsterdam by means of a circular letter asking for material from patients with a number of the syndromes which may include systemic lupus erythematosus, and eighty from normal subjects. The diagnosis is set out in Table I.

The highest titres obtained in the various conditions tested are shown in Table II.

TABLE II
HIGHEST TITRES OBTAINED WITH VARIOUS DIAGNOSES

Test	Diagnosis	Highest Titres Obtained
Loose Body	Systemic lupus erythematosus	1 : 128
	Rheumatoid arthritis	1 : 8
	Rheumatic fever	1 : 8
	Hydralazine syndrome	1 : 4
	Discoid lupus erythematosus	1 : 1
	Malignant hypertension	1 : 1
	Scleroderma	Sporadically positive
	Pleurisy	Sporadically positive
	Idiopathic thrombopenia	Sporadically positive
	Hepatitis	Sporadically positive
	False positive reaction (? syphilis)	Sporadically positive
	Chlorpromazine jaundice	Sporadically positive
	Hashimoto's struma	Sporadically positive
Nucleus Agglutination Inhibition	Thrombosis of the central retinal vein	Sporadically positive
	Systemic lupus erythematosus	1 : 64
	Rheumatoid arthritis	1 : 4
	Rheumatic fever	1 : 4
	Hydralazine syndrome	1 : 4

In one year the presence of the L.E. factor was traced in nineteen patients with the aid of the L.E. test and N.A.I.test. The patients, who had been admitted with varying syndromes, are listed in Table III (opposite).

Case 10 in Table II is described in detail as an example.

TABLE I
RESULTS OF TESTS IN 800 PATIENTS

Diagnosis	No. of Cases	Tests			
		Loose Body	Nuclear Agglutination Inhibition	L.E. Cell	Antiglobulin Fixation
Systemic Lupus Erythematosus	35	34	25	23	11 (of 21)
Rheumatoid Arthritis	208	28	17	10	1 (of 7)
Hydralazine Syndrome	2	2	2	2	0 (of 1)
Rheumatic Fever	22	4	1	—	Unknown
Discoid Lupus Erythematosus	13	4	—	—	Unknown
Malignant Hypertension	7	1	—	—	Unknown
Pleurisy	25	2	—	—	Unknown
Idiopathic Thrombopenia	12	4	—	—	Unknown
Hepatitis	36	1	—	—	Unknown
False Positive Syphilis Reaction	9	1	—	—	Unknown
Scleroderma	3	1	—	—	Unknown
Chlorpromazine-jaundice	3	1	—	—	Unknown
Hashimoto's Struma	1	1	—	—	Unknown
Thrombosis of the Central Retinal Vein	1	1	—	—	Unknown
Other Diagnosis	343	—	—	—	Unknown
Normal Subjects	80	—	—	—	Unknown
Total	800	85	45	35	12 (of 29)

* The Antiglobulin Fixation Test was carried out in the Central Laboratory of the Blood Transfusion Service (Director: Prof. J. J. van Loghem, M.D.).

TABLE III
CLINICAL PICTURE AND DIAGNOSTIC TESTS IN NINETEEN PATIENTS

Case No.	Sex	Age (yrs)	Clinical Manifestations	Tests			
				Loose Body	Nucleus Agglutination Inhibition	L.E. Cell	Antiglobulin Fixation
1	F	83	Arthralgia Hepatomegaly; Acute abdominal symptoms	1 : 2	1 : 1	—	—
2	F	39	Rheumatoid arthritis Pleurisy; Pericarditis; Hepatomegaly; Acute abdominal symptoms	1 : 4	1 : 1	weakly +	—
3	F	65	Pneumonia; Pleurisy; Pericarditis; Microscopic haematuria; Acute abdominal symptoms	1 : 8	1 : 4	+	+
4	F	38	Rheumatoid arthritis; Mitral stenosis; Hepatosplenomegaly; Microscopic haematuria; Leucopenia	1 : 8	1 : 2	—	—
5	F	64	Rheumatoid arthritis; Larynx arthritis; Iridocyclitis; Mitral stenosis; Hepatomegaly; Psychosis; Microscopic haematuria	1 : 2	1 : 1	—	—
6	F	65	Rheumatoid arthritis Iridocyclitis Psychosis Microscopic haematuria	1 : 1	1 : 1	—	—
7	F	57	Arthralgia Chronic hepatitis False positive serological test for syphilis	1 : 16	1 : 4	+	—
8	F	13	Acute hepatitis	1 : 16	1 : 8	+	+
9	F	16	Malignant hypertension Raynaud's syndrome False positive Wassermann reaction Thrombopenia Microscopic haematuria	1 : 1	—	—	—
10	M	59	Rheumatoid arthritis Migratory pneumonia	1 : 128	1 : 32	+	+
11	M	57	Glomerulonephritis Hepatomegaly Bilateral pulmonary fibrosis Macroscopic haematuria	1 : 2	—	—	—
12	M	43	Arthralgia Migratory exanthema Pneumonia Haemolytic anaemia Leucopenia False positive Wassermann reaction Microscopic haematuria	1 : 4	1 : 1	+	+
13	F	69	Liver cirrhosis Microscopic haematuria	1 : 1	—	—	—
14	F	67	Arthralgia Chronic hepatitis (1938) False positive Wassermann reaction	1 : 8	1 : 2	+	—
15	M	58	Rheumatoid arthritis Pleurisy Myocarditis	1 : 128	1 : 64	+	+
16	F	32	Polyarthritits Rheumatic fever	1 : 8	1 : 4	—	—
17	F	32	Pericarditis sicca	1 : 2	1 : 1	+	—
18	F	51	Rheumatoid arthritis Hepatitis Bilateral pleurisy	1 : 16	1 : 8	+	+
19	F	67	Hydralazine therapy (6 mths, 150 mg. per day) Myalgia Bilateral pneumonia Hepatomegaly	1 : 2	1 : 1	+	—

Case 10, a 59-year-old clerk, fell ill in November, 1959, with fever and joint complaints. In the middle of March, 1960, he developed a left-sided pneumonia, which spread to the right side a month later. In 1950 he had had a skin affection on the nose and cheeks.

Examination.—In the Out-patient Clinic the diagnosis of systemic lupus erythematosus was established and the patient was admitted to hospital. Infiltrations were found in both lungs, and the joints were painful on pressure, but did not show any objective abnormalities.

Laboratory Findings.—Hb 11.4 g. per cent.; erythrocytes 4.3 mill.; thrombocytes 400,000/c.mm.; leucocytes 4,600/c.mm.

Normal differentiation.

Erythrocyte Sedimentation Rate: 69 mm. hr.

Thymol Turbidity Test: 12 U.

Bromsulphalein Retention: 18 per cent. after 45 minutes.

Urine: no abnormalities.

Creatinine Clearance: 145 ml. per min.

Protein Spectrum: total protein: 64.7 g./mill., albumin: 20.5 g./mill., α_1 -globulin: 3.8 g./mill., α_2 -globulin: 7.9 g./mill., beta-globulin: 5.9 g./mill., gamma-globulin: 26.6 g./mill.

Serum Glutamic Oxalacetic Transaminase: 32 U.

Hijmans van den Bergh Reaction direct: negative, total: 0.4 U.

Wassermann Reaction: negative.

Serological Tests for Syphilis: negative.

Loose Body Test: positive, 1:128.

Nucleus Agglutination Inhibition Test: positive, 1:32.

L.E. Cell Test: markedly positive.

Antiglobulin Fixation Test: markedly positive.

Result.—The patient was treated with 30 mg. prednisone per day, later with 25 mg. per day. The clinical manifestations (lung infiltrates and joint complaints) disappeared entirely, and the L.B.T. and N.A.I.T. fell to lower values, but still remained strongly positive (1:16 and 1:8 respectively, see Fig. 2). The patient was discharged with continuation of the prednisone therapy.

Discussion

In experiments on the phagocytosis phase of the L.E. cell formation (Formijne and van Soeren 1958), we discovered the lability of the phagocytosis, and observed the stability and constancy of the first phase of the L.E. cell phenomenon, the loose body formation, remained in many changing circumstances.

A study of the literature showed that in the L.E. cell test insufficient conclusive power was attributed to the direct reaction between nuclear material and L.E.-factor. The serological methods are exactly aimed at this reaction, but meet with difficulties in finding the correct indicators. Pathological anatomy is most reliable criterion for the diagnosis of S.L.E.; the haematoxylin staining bodies, chemically and morphologically identical to the loose bodies and inclusion bodies of the L.E. cell phase

Fig. 2.—Clinical course of Case 10.

menon, are also the product of the direct reaction between L.E. factor and nuclear material. In the literature the loose body is not considered to be sufficiently definable, and it has even been stated that free nuclear material and S.L.E. serum cannot form loose bodies (Nathan and Snapper, 1958). This opinion is probably based on the fact that loose nuclei were used in which the free bonds of the nucleoprotein were already blocked in the process of liberating the nucleus from the cell. Another possibility is that this opinion was based on nuclear material of blasts or chick erythrocytes, which indeed, in our experience, do not give rise to a morphologically recognizable loose body formation and do not take part in the nucleoprotein formation.

The rough type loose bodies phenomenon that arises when the amount of L.E.-factor available per nucleus decreases, throws new light on the old controversy regarding the homogeneity of the inclusion body of the L.E. cell. Both van der Schoot (1959) and the present author are of the opinion that there are only quantitative and no qualitative differences.

The phenomenon of nucleus agglutination and its inhibition by serum containing L.E.-factor, which was discovered in our experiments, has already been known in a modified form. Tullis (1953) described nucleoprotein clot formation in old leucocytes, and Lee (1958) noticed that leucocyte agglutination inhibition is a property of S.L.E. sera of sufficient strength. Both authors point out that the leucocytes used for this reaction should be aged. It has appeared to us that the reaction proceeds much more quickly with leucocyte nuclei freed by distilled water. In the weekly titre checks of the L.B.T. and the N.A.I.T. we saw that even rest in bed can produce a fall in the titre. The administration of corticosteroids, however, does this more effectively and may abolish the phenomenon entirely, but the reaction may soon return after their withdrawal. The amount of L.E. factor demonstrable is not an absolute criterion of the severity of the disease process, but only an expression of the individual relationships in the patient.

Checking the titration is valuable in following up the spontaneous course of the disease and its therapeutic management.

The loose body test is reliable from titre 1:1 onwards (massive loose body formation in undiluted serum). The result: "sporadically positive" (20 to 100 rough type loose bodies) should be regarded with some reserve as long as no greater series of results is available.

The results with this test may throw some light on the clinical problems of systemic lupus erythe-

matusus. It appears to be incorrect to persist in separating rheumatoid arthritis with a positive L.E.-cell phenomenon from systemic lupus erythematosus. In all but one of fourteen cases of L.E.-positive rheumatoid arthritis, which were clinically observed by us, abnormalities of liver, kidneys, heart, lungs, serous membranes, eyes, central nervous system, and so on, were demonstrable. These cases, all of which show a protracted course, may therefore be called cases of chronic lupus erythematosus disseminatus (Kurnick, 1956). This opinion is supported by the fact that in such cases the joint capsules and tendon sheaths may show a typical histological picture (Cruickshank, 1959).

The same objection can be advanced for lupoid hepatitis. According to our experience, liver function disturbances (bromsulphalein retention) and hepatomegaly are very frequent manifestations of systemic lupus erythematosus, and the literature shows that, in lupoid hepatitis, symptoms other than the L.E. cell phenomenon are the rule (Mackay, Taft, and Cowling, 1959; Taft, Mackay, and Cowling, 1960; Holman, 1960; Holman and Tomasi, 1960). The constant hypergammaglobulinaemia demonstrated in these cases, which gives rise to anti-complementary syphilis reactions, may also explain the weakly positive or absent L.E. cell phenomenon, since gamma globulin can have a strong anti-complementary activity and may therefore inhibit the phagocytosis. It is in these cases that the discrepancy between the L.E. cell test and the height of the loose body titre is most striking. Lupoid hepatitis thus appears to be a particular form of systemic lupus erythematosus, and lupus hepatitis would therefore be a better name for the condition.

Finally, there are strong indications of the dynamic nature of the anatomical abnormalities in systemic lupus erythematosus. The characteristic changes were not found on autopsy in two patients who had been treated for a long time with high doses of corticosteroids. This points to a continuous formation and breakdown of haematoxylin staining bodies, unless the reaction between antinuclear factor and nuclear material is blocked by high doses of corticosteroids. This is another reason for treating such patients continuously with corticosteroids (Dubois, 1960) to prevent the irreparable damage of the organs, which ultimately develops as a tissue reaction to the products of the anti-nuclear-factor-nucleus-complex.

Summary

Two simple qualitative and quantitative microscopic and macroscopic diagnostic methods (the *Loose Body Test* and the *Nucleus Agglutination*

Inhibition Test) are described. These tests are based on the direct reaction between the L.E. factor and free leucocyte nuclei.

As regards sensitivity, the two methods are superior to the L.E. cell test and the antiglobulin fixation test. The results of an investigation in 800 patients are reported, and the implications of the data obtained are considered.

REFERENCES

- Aisenberg, A. C. (1959). *J. clin. Invest.*, **38**, 325.
 Cruickshank, B. (1959). *Ann. rheum. Dis.*, **18**, 111.
 Dubois, E. L. (1960). *J. Amer. med. Ass.*, **173**, 1633.
 Engelfriet, C. P., Hart, M. van der, and Loghem, J. J. van (1959). *Vox Sang. (Basel)*, n.s. **4**, 485.
 Finch, S. C. and Detre, K. D. (1958). *Clin. Res. Proc.*, **6**, 196.
 Friou, G. J. (1958a). *Proc. Soc. exp. Biol. (N.Y.)*, **97**, 738.
 — (1958b). *J. Immunol.*, **80**, 476.
 — (1958c). *Ann. intern. Med.*, **49**, 866.
 Formijne, P., and Soeren, F. van (1958). *Lancet*, **2**, 1206.
 Holman, H. (1960). *J. Pediat.*, **56**, 109.
 — and Tomasi, T. (1960). *Med. Clin. N. Amer.*, **44**, 633.
 Kurnick, N. B. (1956). *Arch. intern. Med.*, **97**, 562.
 Lee, S. L. (1958). *Blood*, **13**, 778.
 Loghem, J. J. van, Hart, M. van der, Hijmans, W., and Schuit, H. R. E. (1958). *Vox Sang. (Basel)*, n.s. **3**, 203.
 Mackay, I. R., Taft, L. I., and Cowling, D. C. (1959). *Lancet*, **1**, 65.
 Miescher, P. (1955). *Vox Sang. (Basel)*, n.s. **5**, 116.
 Nathan, D. J., and Snapper, I. (1958). *Blood*, **13**, 883.
 Osler, W. (1895). *Amer. J. med. Sci.*, **110**, 629.
 Pollack, A. D. (1959). In G. Baehr and P. Klemperer "Systemic Lupus Erythematosus", (Mount Sinai Hospital Monograph), p. 1. Grune and Stratton, New York.
 Rifkind, R. A., and Godman, G. C. (1957). *J. exp. Med.*, **106**, 607.
 Robineaux, R., Buffé, D., and Kourilsky, R. (1956). *Ann. Inst. Pasteur*, **91**, 109.
 Rohn, R. J., and Bond, W. H. (1952). *Amer. J. Med.*, **12**, 422.
 Schoot, J. B. van der (1959). Thesis, University of Amsterdam.
 Soeren, F. van (1960). Thesis, University of Amsterdam.
 Taft, L. I., Mackay, I. R., and Cowling, D. C. (1960). *Gastroenterology*, **38**, 563.
 Tullis, J. L. (1953). "Blood Cells and Plasma Proteins." Academic Press, New York.
 Worthington, J. W., Baggenstoss, A. H., and Hargraves, M. M. (1959). *Amer. J. Path.*, **35**, 955.

Procédés simples pour déterminer le facteur L.E.

RÉSUMÉ

On décrit deux procédés simples de diagnostic microscopique et macroscopique, qualitatif et quantitatif (Réaction du Corps Libre—*Loose Body Test*—et Réaction d'Inhibition de l'Agglutination du Noyau—*Nucleus Agglutination Inhibition Test*). Ces tests se basent sur une réaction directe entre le facteur L.E. et les noyaux leucocytaires libres.

La sensibilité de ces procédés est supérieure à la recherche des cellules L.E. et à la réaction de fixation de l'antiglobuline. On présente les résultats d'une étude chez 800 malades et on considère les implications des données obtenues.

Métodos simples para determinar el factor L.E.

SUMARIO

Se describen dos sencillos métodos de diagnóstico cualitativo y cuantitativo, microscópico y macroscópico (la Reacción del Cuerpo Libre—*Loose Body Test* y la Reacción de Inhibición de la Aglutinación del Núcleo—*Nucleus Agglutination Inhibition Test*). Dichas pruebas están basadas en la reacción directa entre el factor L.E. y núcleos leucocitarios libres.

La sensibilidad de estos métodos es superior a la busca de células L.E. y a la reacción de fijación de la antiglobulina. Se publican los resultados de una investigación en 800 enfermos y se consideran las implicaciones de los datos obtenidos.