adsorbable on an antigen-antibody complex. Its action is inhibited by anticomplementary substances, such as sodium citrate, EDTA, heparin.

2. The quantitative study shows that the intensity of the leuco-agglutination depends on the amount of complement which is present in the reaction: the excess of complement (more than $^2/_3$ of normal) inhibits the leuco-agglutination; the optimum is equal to $^1/_3$ of the normal level; in the absence of complement the leuco-agglutination is inhibited in 30% of the leuco-agglutinins tested.

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Iso- and Auto-Immunoreactions in Systemic Lupus Erythematosus

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Investigations have shown that iso- and auto-immunoreactions taking place in systemic lupus erythematosus (SLE) are more numerous than in any other disease. Cases with false positive syphilis reactions have long been known. Furthermore, it has been found that patients suffering from this condition are particularly liable to form erythrocytic iso-antibodies, even against substances of weak antigenicity.

The discovery of the L.E.-cell phenomenon by *Hargraves* and co-workers issued in a new epoch in the study of immunological reactions associated with SLE. We would merely recall here that the L.E. factor, responsible for the L.E.-cell phenomenon, was shown to be a gamma-globulin which can be adsorbed specifically on cell nuclei, irrespective of the species or organ from which the cell nuclei originate[5,6]. Cell nuclei can be replaced by nucleoprotein[2,6]. Recently, it was found that desoxyribonucleic acid plays an important part in the antinuclear reactions[2,6,7,9,10,11]. This immunological differentiation of the reactions according to the reactive substances is on the point of yielding further surprising results. We can already say that there are various antinuclear factors, for some of which the specific substrate is nucleoprotein and for others DNA. A short time ago, *Kunkel* and associates at the Rockefeller Institute even succeeded in one case in demonstrating a specific serum reaction with histone[4]. This would mean that the cell nucleus contains at least three specific nuclear antigens or haptens: thus making it possible for antibodies to be formed against nucleoprotein, against the specific nuclear protein histone, and finally against DNA. The L.E.-cell factor is probably a

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mixture of various antibodies, in which sometimes one and sometimes another may be lacking.

The search for further immunoreactions in the serum of patients suffering from SLE did not go unrewarded. Antileucocytic and antithrombocytic antibodies could be found in a large percentage of cases. Finally, it also proved possible to detect antibodies against various tissues, such as kidney, liver, etc. In addition, the fraction II reaction, characteristic of rheumatoid arthritis, may frequently be positive in SLE.

We should like now to discuss the results of this serological research under two headings: firstly, practical consequences as regards diagnosis, and, secondly, pathogenetic aspects.

Table I shows the results of serological tests conducted in our laboratory on 35 patients suffering from SLE. All the tests were repeated several times.

Table I

Results of Seroreactions in 35 Cases of LED (26 ♀, 9 ♂)

		No. cases	%	
L.E. phenomenon		28	80	91 %
Pseudo-L.E. phenomenon		4	11	
Antigl. consumption	+++<	25	71.5	100 %
test with cell nuclei	++	10	28.5	
DNA seroreaction		22	63	
Paratoluene sulfonic acid reaction	++<	15	43	
Platelet antibodies		22	63	
Leucocyte antibodies		19	54	
Fraction II reaction		12	34	

Techniques: L.E.-cell test: Method of *Zimmer and Hargraves*[12]; antiglobulin consumption test with cell nuclei[8]: + = consumption of 1 titre of the antiglobulin serum; ++ = consumption of 2 titres; +++ = consumption of 3 titres. Latex particle agglutination test with DNA and with gamma-globulin[8]; seroreaction with paratoluene sulphonic acid recently described by *Jones and Thomas*[3]*; leucocyte agglutination test with *Dausset's* technique[1], platelet antibodies: antiglobulin consumption test with fresh thrombocytes.

The *L.E.-cell phenomenon* was definitely positive in 80 % of the cases. In 11 % a so-called pseudo L.E.-cell phenomenon was diagnosed, i.e. no completely typical L.E. cells could be observed, but merely phagocytoses of nuclear material which still revealed a certain chromatin structure. In 3 cases, i.e. in 9 %, the L.E.-cell test was regarded as entirely negative.

The *antiglobulin consumption test with cell nuclei* was strongly positive in 71.5 % of the cases and moderately positive in 28.5 %; in no case was it negative. The seroreaction with DNA was positive in 63 %, and the paratoluene sulphonic acid test in 43 %. Thrombocytic antibodies were observed in 63 % of the cases, and leucocytic antibodies in 54 %. The fraction II test was positive in 12 cases (34 %).

These seroreactions represent only a selection of all the possible immuno-

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reactions that can be observed in systemic lupus erythematosus. The findings, however, already suffice to show quite clearly how frequently immunological reactions are in fact demonstrable in this disease. Of diagnostic importance are, primarily, the L.E.-cell phenomenon, the antiglobulin consumption test with cell nuclei, and the fraction II test.

As regards the *L.E.-cell phenomenon*, we, in common with most other authors, have found that it has a very pronounced specificity. Apart from SLE we observed definitely positive L.E.-cell phenomena only in cases of so-called Apresoline disease and rheumatoid arthritis. Where the full L.E.-cell phenomenon is present, therefore, these are the only diseases that have to be considered in the diagnosis. Where the so-called pseudo L.E. phenomenon is found, the possibilities are already far more numerous. Corresponding nucleophagocytic manifestations are quite often observed in hypersensitivity states, carcinomatosis, and rheumatoid arthritis. Thus, a positive pseudo L.E. phenomenon does no more than suggest that systemic lupus erythematosus is a possible diagnosis.

The antiglobulin consumption test is, in our experience so far, the second important laboratory test for the diagnosis of systemic lupus erythematosus. We have found it even more sensitive than the L.E.-cell test. Technique:

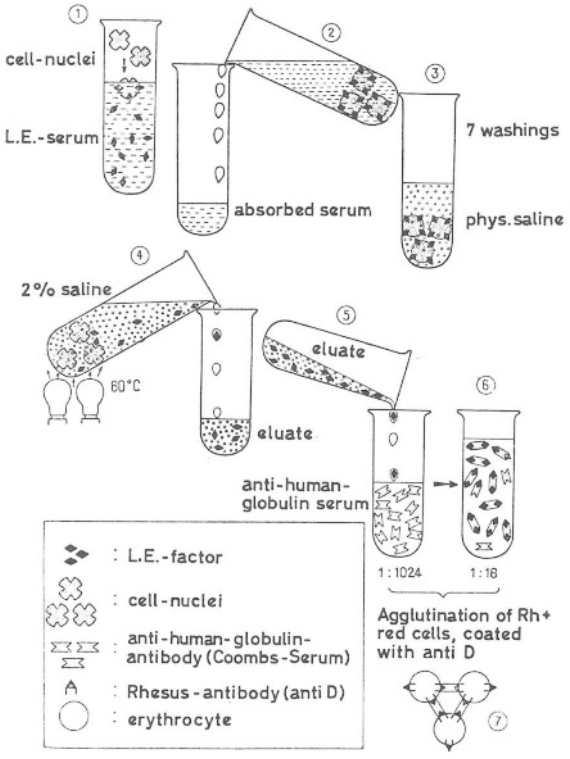

Fig. 1. Schematic representation of the antiglobulin consumption test.

1: The patient's serum (1 ml) is incubated with 10 mg liophilized cell nuclei at 37° C for 30 minutes (a control is always done with normal serum). The cell nuclei are preferably isolated from the thymus of any animal. It is better not tu use human cell nuclei since this may more readily lead to false positive nuclear reactions, especially in the case of antileucocytic antibodes which may react with the residual cytoplasm of human leucocytes. 2 and 3: Following incubation, the nuclei are washed at least seven times with ice-cooled physiological saline. If the patient's serum contained antinuclear factors, the latter will have been firmly deposited on the cell nuclei and will continue to adhere to them undiminished even after many washings. 4: In a further operation, hypertonic saline (0.5 ml 2 % saline) is added and the suspension heated in the water-bath to 60° C during 20 minutes. This will result in the nucleus-specific gamma-globulins becoming detached again from the nuclear substrate. 4 and 5: The suspension is now placed immediately into preheated centrifuging containers and quickly centrifuged. The eluate is again centrifuged and the supernatant is then mixed with equal parts of antihuman globulin serum. 6: If the eluate contains gamma-globulin, this reacts with the anti-gamma-globulin. The consequence of this reaction is a decrease in the titre of the antiglobulin serum. In this particular case, only the titre in respect of immunoglobulin is tested; 7: it is measured with the aid of erythrocyte agglutination, the erythrocytes being charged with Rhesus antibodies.

The results are classified into degrees of intensity, arranged according to whether one or more titres of the antiglobulin serum are lost. The loss of one titre is still almost within the margin of error inherent in the method and is therefore of minor importance. In none of the 35 cases of systemic lupus erythematosus was a titre consumption of only one titre difference observed.

A 2 + result is already much more serious. Out of 400 cases tested in the past year 38 had a 2 + result in the antiglobulin consumption test with cell nuclei: in 10 cases SLE was present, and in 8 it was suspected but could not be conclusively diagnosed. 11 patients had typical rheumatoid arthritis. There were 2 cases of rheumatoid spondylitis. The following conditions were present in one case each according to the clinical diagnosis: collagenosis, status febrilis, cirrhosis of the liver, reticulosis, Hodgkin's disease, coxarthrosis, lumbago.

A result of 3 + or more (tab. II) was observed only 10 times among the 400 patients tested, apart from the cases of systemic lupus erythematosus: rheumatoid arthritis was present in 5 cases, including 2 of Felty's syndrome and one of an atypical, particularly violent form. Three patients suffered from diffuse scleroderma, one had dermatomyositis and another a collagenosis where no closer differentiation was possible (ESR of 90/140 after 1 and 2 hours respectively).

These results show therefore that a full L.E.-cell phenomenon and a strongly positive antiglobulin consumption test with cell nuclei indicate in all probability the presence of systemic lupus erythematosus. In both instances, the only diseases liable to confuse the diagnosis are rheumatoid arthritis, Apresoline-induced lupus erythematosus, or some other collagen disease such as diffuse scleroderma or dermatomyositis. The frequency with which these antinuclear reactions are to be

Table II

Antiglobulin Consumption Test with Cell Nuclei +++ Result in 400 Cases with Various Diseases

		No. cases
LED		25
Other diseases		10 (2.5%)
Rheumatoid arthritis	5	
Diffuse scleroderma	3	
Dermatomyositis	1	
"Collagen disease"	1	

expected in rheumatoid arthritis is shown by table III which summarizes the data on 100 serologically tested cases: the characteristic test for this disease is the fraction II reaction which yielded positive results in 78 % of the cases. Other authors have had up to 90 % positive tests. In 6 cases there was a definite L.E.-cell phenomenon which could not be distinguished from a genuine one. 12 cases showed a strongly positive pseudo-L.E. phenomenon. The antiglobulin consumption test with cell nuclei was weakly positive 23 times; this, of course, is only of limited importance since a 1 + result is within the method's margin of error and is observed in approximately 12 % of the control sera. On the other hand, a 2 + was obtained in 11 cases and a 3 + in 5. Finally, the seroreaction with DNA was positive in 6 of these 100 cases.

Table III

Results of Seroreaction in 100 Cases of Rheumatoid Arthritis

		No. cases positive result	negative result
Fraction II reaction		78	22
L.E. phenomenon		6	82
Pseudo-L.E. phenomenon		12	
Antigl. cons. test	(+	23)	
with cell nuclei	++	11	84
	+++	5	
DNA reaction		6	94
Paratoluene sulfonic acid reaction	++<	9	91

If we consider the clinical manifestations of rheumatoid arthritis and systemic lupus erythematosus, these serological findings are no longer so surprising. It is often very difficult for the clinician to decide whether the patient has atypical rheumatoid arthritis or systemic lupus erythematosus. Serological tests thus confirm clinical experience that these two diseases are very close. We do not, however,

believe that we are dealing here with two forms of the same disease. Various facts, which we cannot discuss separately here, support the assumption that two distinct nosological entities are involved.

And so we come to the question of the importance of the immunological reactions for the *pathogenesis* of systemic lupus erythematosus.

First of all, the various immunological reactions shed new light on the known fact that systemic lupus erythematosus is a disease which tends to result in sensitization against all possible antigens. Whether this immunological diathesis is a consequence of the disease or whether it is pre-existent cannot be said with certainty. Nevertheless, the fact that the incidence of the disease is high in some families – a particularly instructive example has been described in Sweden by *Waldenström* and co-workers – suggests that this diathesis pre-exists. The family in question showed a predisposition to hyperglobulinaemia, which indicates that the tendency to form antibodies is an individual, pre-existent characteristic that favours the contraction of systemic lupus erythematosus.

Among the various seroreactions there are two categories which are of particular assistance in evaluating the pathogenesis: the antinuclear reactions and the fraction II test.

As regards the antinuclear reactions, various facts seem to indicate that these not only represent an *in vitro* phenomenon, but somehow reflect the nature of the disease. Do not the haematoxylin-bodies bear witness to a pathological process of cellular disintegration taking place during life? Several authors even suppose that the renal changes are due in part to cellular degradation processes. It would be tempting to conclude that these cell changes, which indeed correspond in principle to those of the L.E.-cell phenomenon observed *in vitro*, are to be ascribed to the effect of anti-nuclear antibodies, i.e. that the disease is one of auto-aggression in the real sense of the word. We are, however, still a long way from being able to prove the truth of such a view. On the contrary, there are various reasons for believing that the antinuclear antibodies do not exert a pathogenic effect. Firstly, it is striking that the serum titre of these antibodies does not reflect the degree of intensity of the disease. Secondly, we know that the L.E. factor can be transmitted diaplacentally to the foetal organism, without the infant suffering any harm or showing even the slightest signs of disease. Another argument may be seen in the fact that the L.E. factor cannot be absorbed on intact cells which makes it difficult to admit that an antinuclear reaction takes place in living cells *in vivo*. Finally, cutaneous lupus erythematosus, which is nosologically the same disease in principle, is not accompanied by antinuclear antibodies. A further argument against antinuclear factors having a specifically pathogenic significance can be seen in the fact that they are found in 5–15 % of cases of rheumatoid arthritis although the patients concerned do not contract systemic lupus erythematosus in a corresponding number of cases. The question of rheumatoid arthritis developing into systemic lupus erythematosus has not yet been fully elucidated, but such an occurrence seems to be at all events rare and bears no relation to the incidence of antinuclear reactions in rheumatoid arthritis. It is therefore quite possible that the antinuclear factors are not the cause

but the consequence of cellular changes taking place during life, or even the consequence of a hetero-sensitization with bacterial or viral nucleoprotein.

As for the *fraction II reaction*, the fact that it is observed in a considerable number of cases of lupus erythematosus under-lines the immunological relationship between this disease and rheumatoid arthritis. However, according to *Nana Schwartz*, the factor responsible for the reaction does not seem to be completely identical in the two diseases.

As regards the significance of this reaction for the pathogenesis of the disease, this is a matter about which we know nothing. Together with *Alpstäg and Strässle* we were able to show that patients with a positive fraction II reaction do not have an increased turnover of gamma-globulin[11a].

In conclusion, therefore, we may say that although a number of immunological reactions have been observed in the serum of patients suffering from systemic lupus erythematosus and although the nature of some of these reactions has been largely clarified, their significance for the pathogenesis of the disease is still very obscure. On the other hand, certain of these reactions have become of great practical importance for the diagnosis of systemic lupus erythematosus and related collagen diseases.

References

1. *Dausset, J.:* Immunohématologie clinique et biologique (Flammarion, Paris 1956).
2. *Holman, H. R.* and *Kunkel, H. G.:* Affinity between the L.E. serum factor and cell nuclei and nucleoprotein. Science *126:* 162 (1957).
3. *Jones, K. K.* and *Thompson, H. E.:* Evaluation of simple precipitation test for systemic lupus erythematosus. J. amer. med. Ass. *166:* 1424 (1958).
4. *Kunkel, H. G.:* Personal communication.
5. *Miescher, P.* et *Fauconnet, M.:* L'absorption du facteur «L.E.» par des noyaux cellulaires isolés. Experientia *10:* 252 (1954); Les constituants antigéniques du leucocyte polynucléaire et leur importance clinique. Schweiz. med. Wschr. *84:* 1036 (1954).
6. *Miescher, P.:* Zur Serologie des L.E.-Phänomens. Hautarzt *8:* 502 (1957).
7. *Miescher, P.* and *Strässle, R.:* New serological methods for the detection of the L.E.-factor. Vox Sang. *2:* 283 (1957).
8. *Miescher, P.:* Le lupus érythémateux disséminé: Le phénomène L.E. et les séro-réactions anti-substances nucléaires. 3e Congr. int. Allergol., p. 557 (Flammarion, Paris 1958).
9. *Polli, E.; Celeda, F.* e *Cepellini, R.:* A proposito di un fattore serico del lupus eritematoso sistematico, reagente con l'acido desossipentosonucleico. Boll. Ist. sieroter. milan. *55:* 910 (1957).
10. *Seligmann, M.:* Mise en évidence dans le sérum de malades atteints de lupus érythémateux disséminé d'une substance déterminant une réaction de précipitation avec l'acide désoxyribonucléique. Comptes rendus *245:* 243 (1957).
11. *Seligmann, M.* et *Robineaux, R.:* Induction du phénomène L.E. par l'anticorps anti-acide-désoxyribonucléique isolé à partir du sérum de malades atteints de L.E.D. Comptes rendus *246:* 1472 (1958).

11a. *Strässle, R.; Alpstäg, H.* und *Miescher, P.:* 1st int. Symp. Immunopathology, p. 373 (Schwabe, Basel 1959).

12. *Zimmer, F. E.* and *Hargraves, M. M.:* The effect of blood coagulation on L.E. cell formation. Proc. Staff Meet., Mayo Clin. *27:* 424 (1952).