

SERUM PROTEINS, TRACE ELEMENTS, HEMOSTASIS PARAMETERS,  
FOOD ANTIGENS AND RELATED VARIABLES INVOLVED  
IN THE PATHOGENESIS OF CHRONIC CUTANEOUS LUPUS ERYTHEMATOSUS

A preliminary study on 28 cases following Kozak treatment

SERUM PROTEINS, TRACE ELEMENTS, HEMOSTASIS PARAMETERS,  
FOOD ANTIGENS AND RELATED VARIABLES INVOLVED  
IN THE PATHOGENESIS OF CHRONIC CUTANEOUS LUPUS ERYTHEMATOSUS

A preliminary study on 28 cases following Kozak treatment

By

Gruia Ionescu, Pavel Kozak, Anca Rotaru,  
Cristian Rarinca, Stefan Hulea, Dan Teofil,  
Ion Brad, Marian Popa, Magda Predescu

Fundeni Clinical Hospital, Dermatology Department, Bucharest, 7000, Romania

SUMMARY

A complex study of serum protein fractions, trace elements, hemostasis parameters, food antigens and other related variables was performed in 28 chronic DLE patients with more than 10 year history disease. Serum total protein and serum albumin were significantly decreased in the DLE group as compared with a blood banking donors control group.

A deficient liver protein synthesis and the presence of anti-albumin anti-bodies in 50 pc of the DLE sera are thought to be the most important factors responsible for this phenomenon. Serum  $\alpha_1$  trypsin inhibitor and serum  $\alpha_2$  macroglobulin exhibited increased values in 80 pc and respectively 85,5 pc of the DLE sera; this fact is attributed to their compensatory antiplasminic activity which generates increased levels of fibrinogen/fibrin splitting products.

Serum caeruloplasmin and serum copper showed slightly raised levels in 35 pc and respectively 25 pc of the DLE sera. The compensatory antioxidant activity of this cuproprotein seems to be of great value in the damaged skin areas.

Serum zinc, a copper antagonist, exhibits levels under the normal range in 45 pc of the tested DLE sera, missing the host of its antiinflammatory action. Serum haptoglobin was very significantly increased in DLE sera, 55,5 pc of the DLE values ranging over the normal limits. This increase copes with the host necessity to bind larger hemoglobin amounts in view of the higher protective peroxidase activity of this complex at the injured sites where peroxide radicals are accumulating. Serum hemopexin raises close parallelly with haptoglobin in DLE sera in a compensatory effect to bind the hem necessary to the impaired hemoglobin synthesis.

Serum transferrin and serum iron are maintained within the normal range while serum complement  $C_3$  ( $\beta_{1A}$ ), shows a significant decrease in 44,44 pc of the cases, corresponding to its consumption in the immune complexes of the lupic skin and of the blood vessel wall deposits.

Serum immunoglobulins showed increased values, especially IgA and IgM. A protective significance is attributed as it is also suggested by their levels at the externement. IgE showed detectable levels in 31,4 pc of the DLE patients suggesting an allergic (basophil mediated ?) mechanism of the disease in a number of cases. This assumption is supported by the detection of the anti-food antibodies in 25-33 pc of DLE sera.

A low incidence of antinuclear and anti-smooth muscle antibodies was noted (10,7 and 7,1 pc respectively) proving the non-systemic involvement of the disease.

Platelet agregation was significantly increased in all 12 DLE cases tested suggesting the first step in an intravascular disseminated coagulation pattern.

The antigen-antibody complexes continuously supplied by the presence of different food antigens, are supposed to be the main inducers of this hyperaggregability in a number of cases. The albumin-antialbumin antibody complexes could also be involved.

A compensatory high fibrinolytic mechanism is demonstrated and a tissular basophil mediated allergic mechanism with the participation of IgE is also suggested.

The high eosinophil levels encountered in 32 pc of the cases are also discussed. The complex Kozak treatment applied with very favorable clinical effects to the DLE patients, resulted in a dramatic recovery of the most biological parameters too, and moreover it stimulated certain serum factors with protective significance.

## INTRODUCTION

The significant clinical results obtained by the application of Kozak method in the treatment of chronic discoid lupus erythematosus (DLE) repellent to current therapies /1, 2/ stimulated a complex research programme on the internal parameters aiming both to follow the effects of the administered therapy and to clarify certain aspects of the disease pathogenesis.

We report here the preliminary results in the study of certain serum proteins, microelements, hemostasis factors as well as other connected variables, some of them being yet uninvestigated in chronic DLE according to the data available in the literature.

## MATERIAL AND METHODS

### CHRONIC DLE PATIENTS

A group of 28 patients, 18 males and 10 females admitted to the Dermatology Department of the Fundeni Clinical Hospital with chronic DLE were considered in our study.

The following major clinical forms were recorded:

I. Extensive chronic discoid lupus erythematosus - 18 patients with a mean history disease of 13 years. Among the antecedents, tuberculosis in 16 pc of these cases and important digestive troubles in other 22 pc, were recorded.

All patients have followed continuous or intermittent antimalarial treatments associated with topical corticotherapy, vitamins and tuberculostatics, respectively.

Repellent to any current treatment in the last two years.

II. Chronic DLE (as such) - 10 patients with a mean history disease of 10 years. Among the antecedents, 33 pc with first and second degree hypertension, 33 pc tuberculosis, and other 10 pc with diabetes, were recorded.

Previous treatments as above.

Repellent to any current treatment in the last two years.

The mean age of patients ranged between 28 and 49 years. All of them exhibited a normal renal clearance, an ESR value under 60 mm/h; lupic cells were absent. ANA index positive in only three cases (10.7 pc) and no other sign of systemic clinical involvement noted.

All patients were subjected to Kozak therapy on an average period of 56 days.

#### CONTROL GROUP

A group of 16 apparently health blood banking donors (8 males and 8 females) between 22 and 48 years old was used as control.

The blood samples were kindly supplied by the Institute of Hematology of Bucharest.

#### BLOOD SAMPLES

Total blood samples collected from DLE patients and controls, either heparinized or treated with  $\text{Na}_2$  EDTA or  $\text{Na}_2$  citrate were subjected to the usual hematologic and biochemical tests.

Other indexes as: platelet aggregating capacity, and fibrinogen/fibrin splitting products were also estimated.



## SERUM SAMPLES

Serum samples obtained after the coagulation of total blood were stored in 2 ml aliquots at  $-20^{\circ}\text{C}$ .

## SERUM PROTEIN FRACTIONS

Albumin,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$  and  $\gamma$  globulin were determined by paper electrophoresis and expressed as pc of total protein. Albumin was also chemically estimated in V. Babeş Institute.

SERUM  $\alpha_1$ -TRYPSIN INHIBITOR,  $\alpha_2$ -MACROGLOBULIN,  $\alpha_2$ -HS-GLICO-PROTEIN, HEMOPEXIN, TRANSFERRIN, and IgE were estimated by radial immunodiffusion method using Behringwerke M. - Partigen plates (L-Partigen for IgE), and appropriate Behring standard sera.

CERULOPLASMIN was estimated by Ravin method using p-phenylendiamin as substrate.

SERUM COMPLEMENT  $\text{C}_3$  ( $\beta_{1A}$ ), IgG, IgA and IgM were tested using immunodiffusion plates manufactured by the Cantacuzino Institute in Bucharest and by a standardised rocket immunoelectrophoresis method (Laurell). A good correlation of results was achieved by these two methods.

THE PEROXIDASE ELECTROPHORETIC PATTERN of hemoglobin-haptoglobin complexes in blood lysates was performed in acrylamide-agarose gels in a tris-glycine running buffer pH 8.3. Staining with benzidine in the presence of  $\text{H}_2\text{O}_2$  and a sodium-acetate buffer pH 5.0.

## IMMUNOGLOBULINS BIOLOGIC ACTIVITY

Antinuclear antibodies and anti-smooth muscle antibodies were estimated in our laboratory and in Victor Babeş Institute by an indirect immunofluorescence method using a goat anti-human  $\gamma$ -globulin serum. Slides were read using a HB 200 UV lamp incorporated in an I. O. R. M. C. 5A microscope.

ANTI-HUMAN-ALBUMIN ANTIBODIES were determined using a double diffusion method (Ouchterlony) in agarose gel and a passive hemmagglutination method introduced by a research group in V. Babeş Institute led by dr. R. Lenkey. A human glutaraldehyde polymerised albumin was used as antigen in both techniques.

## ANTI-FOOD-ANTIBODIES

Antibodies directed against certain food antigens were demonstrated in the DLE patient sera using the Ouchterlony method in agarose gels. Purified egg albumin, total egg albumen and a lamb meat extract served as antigens in concentrations of 30 mg/ml and 10 mg/ml respectively.

SERUM TRACE ELEMENTS (Cu, Zn, Fe) were investigated in the Hygiene and Epidemiology Laboratory of the Elias Hospital Bucharest, on a number of 20 DLE patient sera and controls by atomic absorption spectrophotometry.

A Pye Unicam SP 191 atomic absorption spectrophotometer was used. The 1:9 bidistilled water diluted sera were directly aspirated into an air-acetylene flame and read for copper and zinc at 324.8 and 213.9 nm respectively, against appropriate standard solutions.

Iron was read at 243.9 nm in the supernatant of sera precipitated with 20% trichloroacetic acid in the presence of HCl 4 N, against appropriate standard solutions.

SERUM CALCIUM was estimated chemically.

#### TRACE ELEMENTS IN SKIN BIOPSIES

Biopsic samples from affected areas fixed in 10% formol were investigated for Copper, Zinc, Iron and Calcium using special stainings with Na diethyl dithiocarbamate, ditizon, K ferrocyanide, and Luxol Fast Blue MBS respectively.

#### PLATELET AGGREGATION

Platelet rich plasma from blood samples on Na citrate were investigated for platelet aggregation in 12 DLE patients. Aggregometer determinations were performed by adding appropriate concentrations of ADP ( $< 1 \mu\text{mol/l}$ ) or collagen, as inducers of the platelet aggregation; values over 20 pc absorbance reduction were considered pathologically.

#### FIBRINOGEN AND FIBRINE - SPLITTING PRODUCTS

Fibrinolysis products were estimated in 12 DLE patients by a precipitation reaction with a Staphiloclumping reagent (Stago -France). Values over 10  $\mu\text{g/ml}$  were considered abnormal.

Platelet aggregation and FSP were evaluated at the Hematology Department of Fundeni Clinical Hospital.

All the above tests were performed in chronic DLE patients at the admission and at the end of the hospitalisation period.

Results were compared with those belonging to the control group (16 blood banking donors).



## RESULTS AND COMMENTS

### I. SERUM PROTEINS

Sera from all chronic DLE patients showed significant changes in the protein pattern before the admission.

In most of cases administration of Kozak therapy resulted in an obvious amelioration of the proteic balance, fact which was positively correlated with the clinical picture.

#### 1. SERUM TOTAL PROTEINS AND THE ELECTROPHORETIC PATTERN

TOTAL PROTEINS estimated by the biuret method were at the lower limit of the normal range (Fig. 1), 32 pc of the patients showing levels under 6.4 g/dl.

As renal impairment was noted in none of the patients (normal creatinine clearance, albuminuria absent, normal sediment) the lower protein levels suggest the occurrence of a diminished synthesis in the liver cells and/or an imbalanced protein intake.

A disturbed intestinal splitting of the proteic food could also be involved (see below the high levels of  $\alpha_1$ -antitripsin) in a malabsorption phenomenon, the dispeptic troubles found in 22 pc of the patients supporting this idea.

Kozak therapy applied to these patients increased the total protein content in a significant proportion. ( $p_2 < 0,05$ ).

THE SERUM ELECTROPHORETIC PROFILE shows significant changes in all patients before admission as compared with the control group.

Serum albumin showed a significant decrease in 89,3 pc of the chronic DLC patients (levels ranging between 38-53 pc of total protein). The levels in the control group ranged between 52-64 pc.

# SERUM TOTAL PROTEIN IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS BEFORE AND AFTER KOZAK THERAPY. (PRELIMINARY RESULTS).

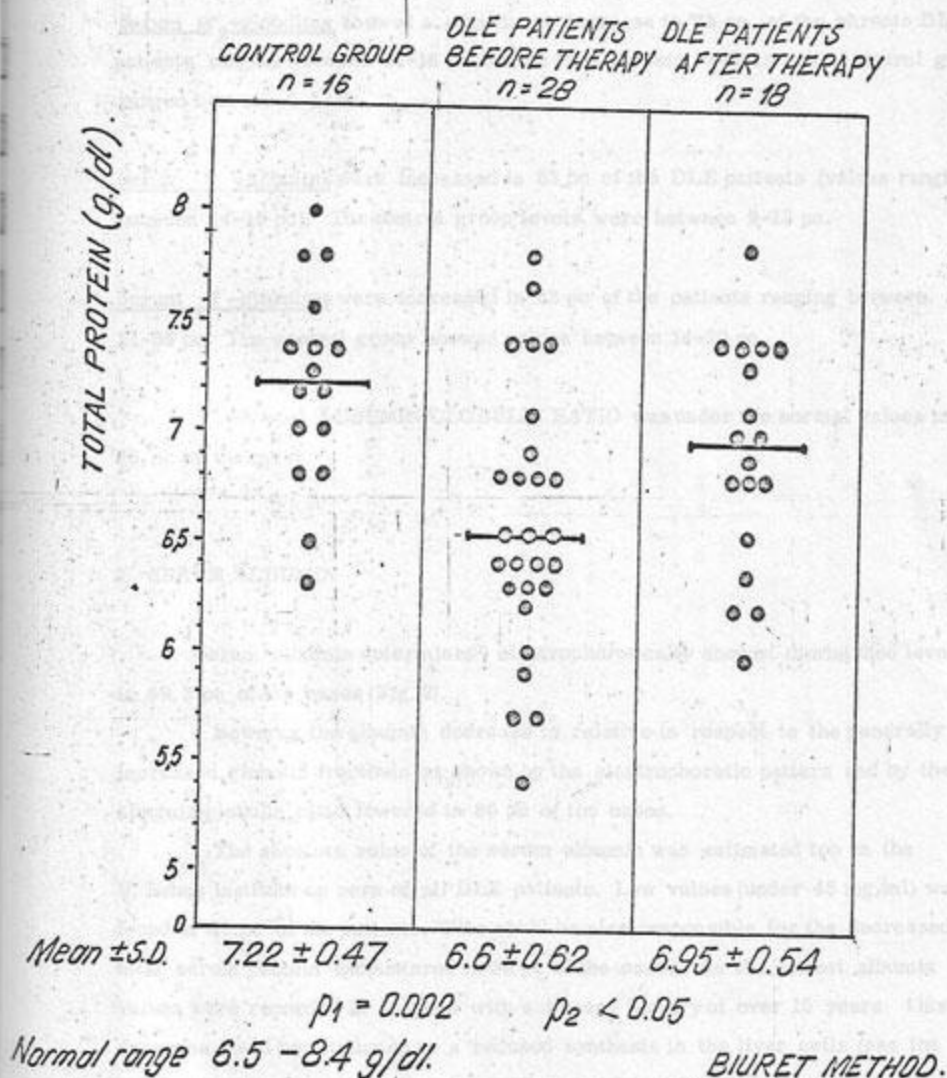


Fig. 1

Serum  $\alpha_1$ -globulins were maintained at the upper limit of the normal range (4-7 pc). The levels in the control group ranged between 2-6 pc.

Serum  $\alpha_2$ -globulins showed a significant increase in 72 pc. of the chronic DLE patients ranging between 11-18 pc. of the total protein. Values in the control group ranged between 6-10 pc.

Serum  $\beta$ -globulins were increased in 63 pc of the DLE patients (values ranging between 14-19 pc). The control group levels were between 9-13 pc.

Serum  $\gamma$ -globulins were increased in 63 pc of the patients ranging between 21-33 pc. The control group showed values between 14-20 pc.

The total ALBUMIN/GLOBULIN RATIO was under the normal values in 86 pc of the cases.

## 2. SERUM ALBUMIN

Serum albumin determined electrophoretically showed diminished levels in 89.3 pc of the cases (Fig. 2).

However the albumin decrease is relative in respect to the generally increased globulin fractions as shown by the electrophoretic pattern and by the albumin/globulin ratio lowered in 86 pc of the cases.

The absolute value of the serum albumin was estimated too in the V. Babeş Institute on sera of all DLE patients. Low values (under 45 mg/ml) were found in 43 pc of the patients. This could be also responsible for the decreased total serum protein encountered in 32 pc of the cases. As the lowest albumin values were recorded in patients with a disease history of over 15 years this decrease could be attributed to a reduced synthesis in the liver cells (see the previous long-term antimalarial treatments with hepatotoxic effects) and also to the high incidence of the anti-albumin antibodies found in chronic DLE patients (see below).

# SERUM ALBUMIN IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS BEFORE AND AFTER KOZAK THERAPY. (PRELIMINARY RESULTS)

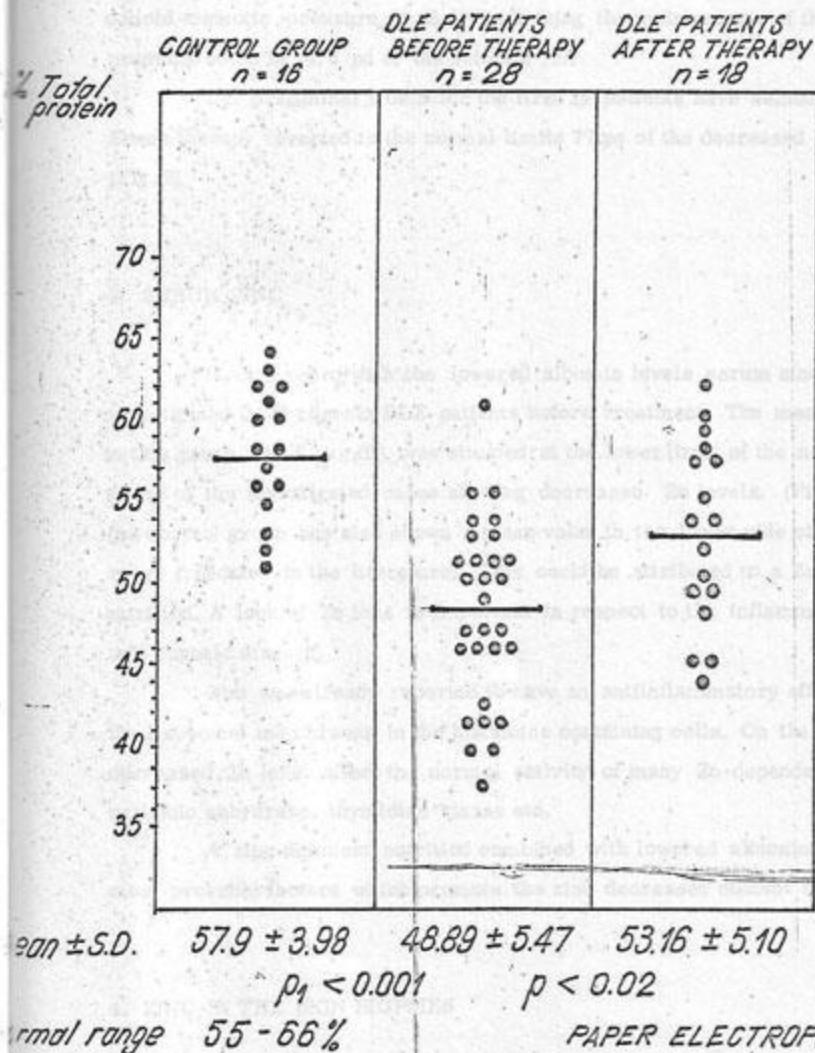


Fig. 2

Serum albumin decrease brings about a lower transport potency and level of important cations as Zn, Cd, Ca (see below) together with a diminished capacity for the neutralization of toxic compounds as Hg, bilirubin, drugs. On the other hand a lower albumin content induces severe alterations in the plasma colloid-osmotic pressure partially explaining the maintenance of the increased arterial pressure noted in 14.3 pc of the patients [2].

Our preliminary data for the first 18 patients have demonstrated that Kozak therapy reverted to the normal limits 77 pc of the decreased albumin values. (Fig. 2)

### 3. SERUM ZINC

In connection with the lowered albumin levels serum zinc levels were investigated in 20 chronic DLE patients before treatment. The mean value obtained in this group ( $32.5 \mu\text{g/dl}$ ) was situated at the lower limit of the normal range, 45 pc of the investigated cases showing decreased Zn levels. (Fig. 3). However the control group has also shown a mean value in the lower side of the normal range (reported in the literature). This could be attributed to a Zn deficient nutrition. A lack of Zn ions is important in respect to the inflammatory aspects of this chronic disease.

Zinc was already reported to have an antiinflammatory effect by stabilising the lysosomal membranes in the histamine containing cells. On the other hand the decreased Zn level affects the normal activity of many Zn-dependent enzymes as carbonic anhydrase, thymidine kinase etc.

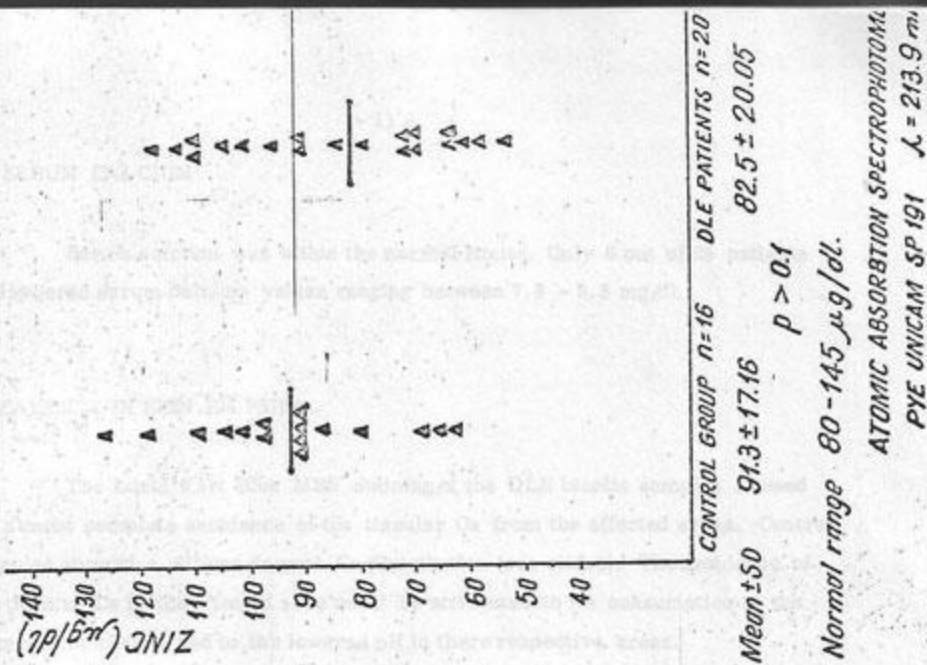
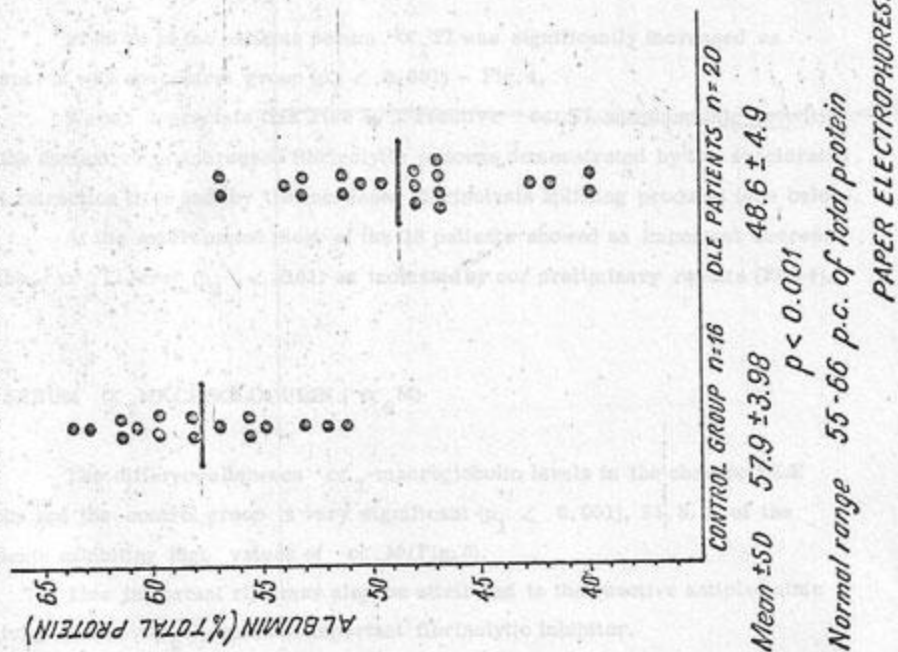
A zinc deficient nutrition combined with lowered albumin levels are the most probable factors which promote the zinc decreased content of DLE sera.

### 4. ZINC IN THE SKIN BIOPSIES

No detectable Zn was evidenced in the affected areas using a ditizon staining method. Control biopsies showed only small amounts of red granular Zn in the upper derm.



# SERUM ALBUMIN AND SERUM ZINC IN CONTROLS AND IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS. (PRELIMINARY RESULTS).



## 5. SERUM CALCIUM

Serum calcium was within the normal limits. Only 6 out of 28 patients had lowered serum calcium values ranging between 7.8 - 8.8 mg/dl.

## 6. CALCIUM IN SKIN BIOPSIES

The Luxol Fast Blue MBS staining of the DLE bioptic samples showed an almost complete avoidance of the tissular Ca from the affected areas. Control samples showed a diffuse dermal Ca distribution (see slides). The avoidance of the dermal Ca in the affected area could be attributed to its consumption in the immune complexes and to the lowered pH in there respective areas.

## 7. SERUM $\alpha_1$ -TRYPSIN-INHIBITOR ( $\alpha_1$ TI)

In 80 pc of the patients serum  $\alpha_1$ TI was significantly increased as compared with the control group ( $p_1 < 0.001$ ) - Fig. 4.

We can appreciate this rise as a reactive  $\alpha_1$ TI antiplasminic activity in the context of an increased fibrinolytic process demonstrated by the accelerated clot retraction time and by the increased fibrinolysis splitting products (see below).

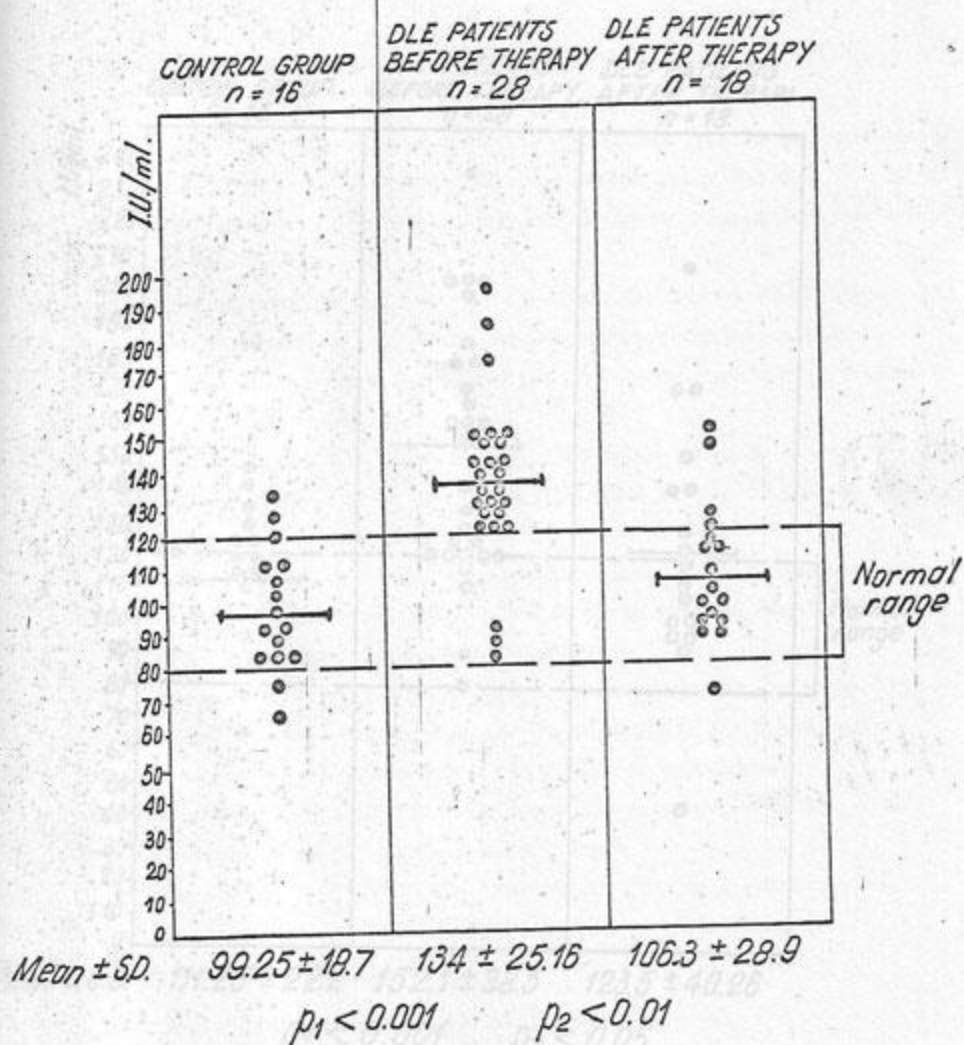
At the extenement most of the 18 patients showed an important decrease in the  $\alpha_1$ TI level ( $p_2 < 0.01$ ) as indicated by our preliminary results (Fig. 4).

## 8. SERUM $\alpha_2$ MACROGLOBULIN ( $\alpha_2$ M)

The difference between  $\alpha_2$ -macroglobulin levels in the chronic DLE group and the control group is very significant ( $p_1 < 0.001$ ), 85.5 pc of the patients exhibiting high values of  $\alpha_2$ M (Fig. 5).

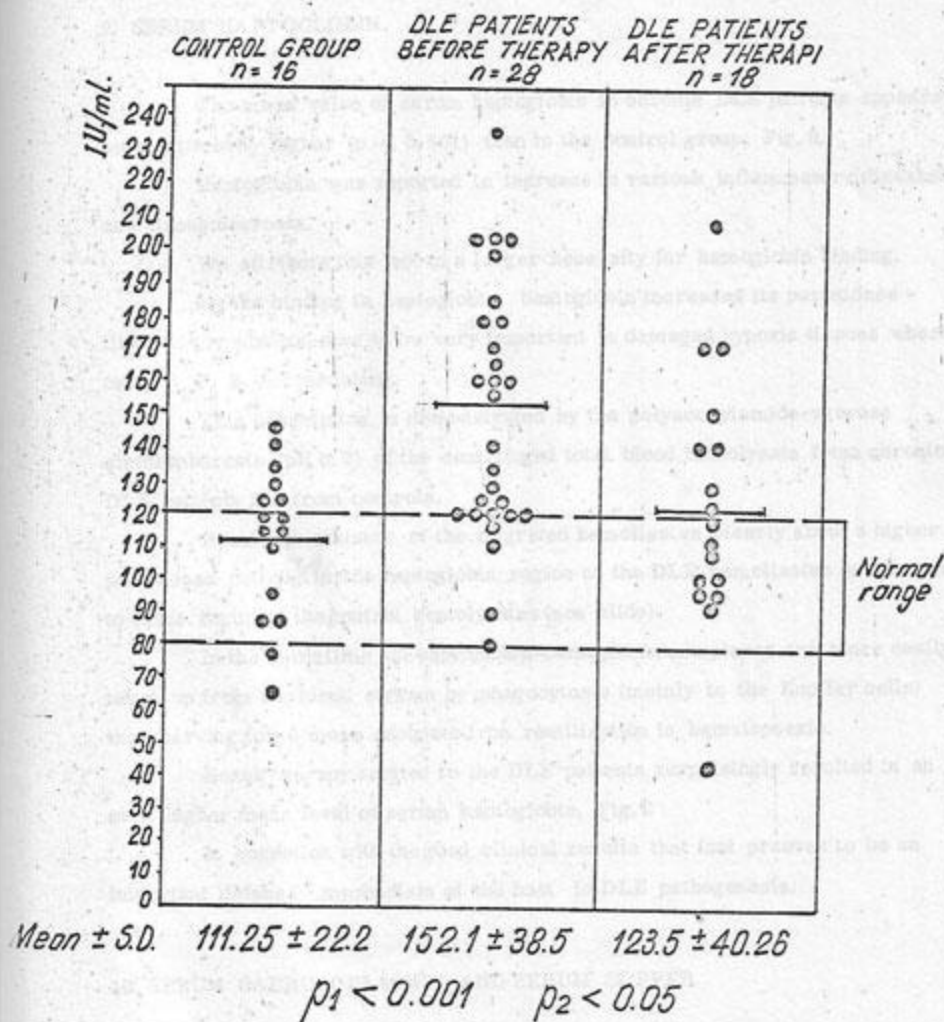
This important rise may also be attributed to the reactive antiplasminic activity of the  $\alpha_2$ M, another important fibrinolytic inhibitor.

SERUM  $\alpha_1$ -TRYPsin-INHIBITOR IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS BEFORE AND AFTER KOZAK TREATMENT (PRELIMINARY RESULTS).



RADIAL IMMUNODIFFUSION METHOD.

SERUM  $\gamma_2$ -MACROGLOBULIN IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS BEFORE AND AFTER KOZAK TREATMENT. (PRELIMINARY RESULTS).



At the extirpation 55.5 pc of the  $\alpha_2$ M levels were reverted into the normal range.

## 9. SERUM HAPTOGLOBIN

The mean value of serum haptoglobin in chronic DLE patients appears to be significantly higher ( $p < 0.001$ ) than in the control group. Fig. 6.

Haptoglobin was reported to increase in various inflammatory diseases and tissue necrosis.

We attribute this fact to a larger necessity for hemoglobin binding.

By the binding to haptoglobin, hemoglobin increases its peroxidase-like activity which seems to be very important in damaged hypoxic tissues where toxic  $H_2O_2$  is accumulating.

This assumption is demonstrated by the polyacrylamide-agarose electrophoresis (pH 8.3) of the centrifuged total blood hemolysate from chronic DLE patients and from controls.

Benzidine staining of the migrated hemolysates clearly show a higher peroxidase activity in the haptoglobin region of the DLE hemolysates as compared to same region of the control hemolysates (see slide).

In the meantime the haptoglobin-hemoglobin complexes are more easily taken up from the blood stream by phagocytosis (mainly in the Kupffer cells) thus serving for a more adequate iron reutilization in hematopoiesis.

Kozak therapy applied to the DLE patients surprisingly resulted in an even higher mean level of serum haptoglobin, Fig. 6

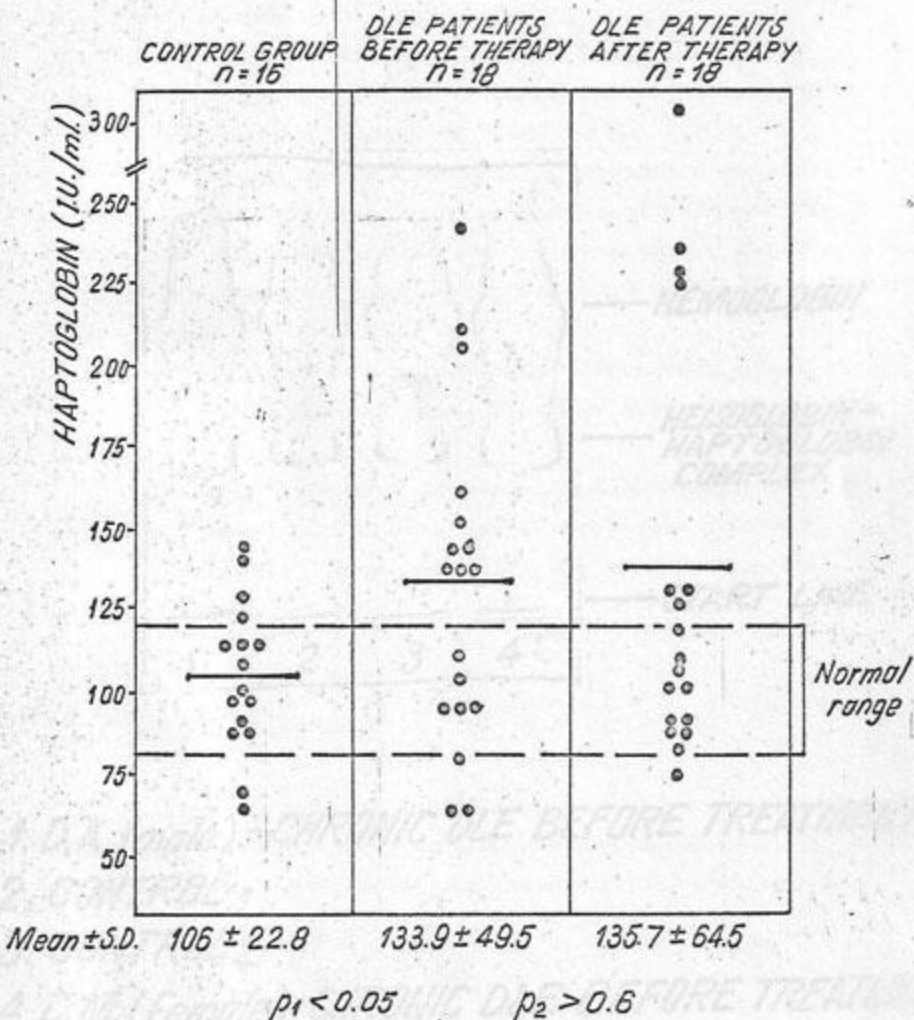
In connection with the good clinical results that fact proves to be an important defense mechanism of the host in DLE pathogenesis.

## 10. SERUM CAERULOPLASMIN AND SERUM COPPER

Caeruloplasmin, the main copper carrier in serum, estimated by Ravin method in 20 chronic DLE patients sera shows a mean value in the normal range



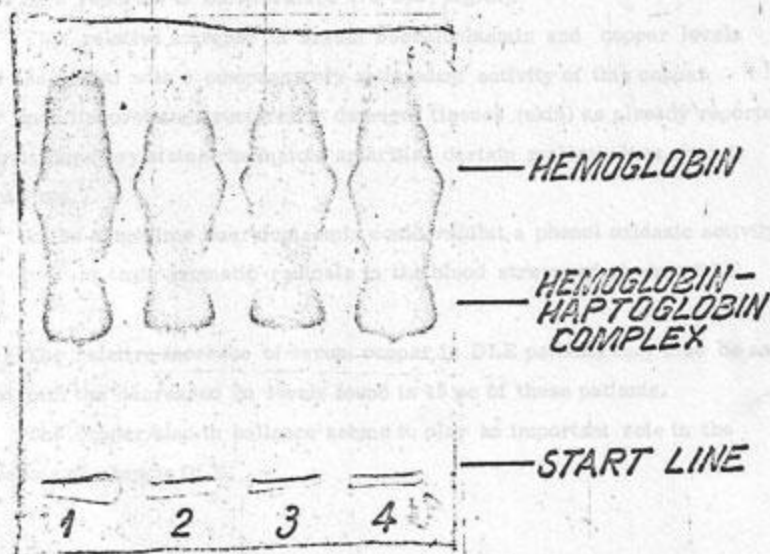
# SERUM HAPTOGLOBIN IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS BEFORE AND AFTER KOZAK TREATMENT (PRELIMINARY RESULTS).



Normal range 80-120 I.U./ml.

RADIAL IMMUNODIFFUSION

# PEROXIDASE ELECTROPHORETIC PATTERN OF BLOOD LYSATES IN CHRONIC DLE PATIENTS.



1. D.A. (male) - CHRONIC DLE BEFORE TREATMENT
2. CONTROL<sub>1</sub>
3. CONTROL<sub>2</sub>
4. C.M. (female) - CHRONIC DLE BEFORE TREATMENT

POLYACRYLAMIDE-AGAROSE GEL ELECTROPHORESIS  
TRIS-GLYCINE BUFFER pH 8.3  
BENZIDINE STAINING

and only slightly higher than in the control group ( $p > 0.1$ ) (Fig. 7).

However in 35 pc of the cases the recorded levels were above the upper limit of the normal value showing a good correlation with the slightly raised copper levels recorded in 25 pc of the cases. (Fig. 7).

Serum copper in chronic DLE patients was significantly higher as against the control group ( $p < 0.05$ ) although the mean value belongs to the normal range reported in the literature (70-140  $\mu\text{g/dl}$ ).

This relative increase in serum caeruloplasmin and copper levels may be associated with a compensatory antioxidant activity of this copper carrier upon lipoprotein structures in damaged tissues (skin) as already reported in other inflammatory states (rheumatoid arthritis, certain malignancies, chronic infections etc.).

In the meantime caeruloplasmin could exhibit a phenol oxidase activity against different toxic aromatic radicals in the blood stream of chronic DLE patients.

The relative increase of serum copper in DLE patients may also be associated with the decreased Zn levels found in 45 pc of these patients.

The copper/zinc in balance seems to play an important role in the pathogenesis of chronic DLE.

## 11. COPPER IN SKIN BIOPSIES

Copper was found neither in DLE skin biopsies from affected areas nor in normal skin biopsies as demonstrated with a diethyldithiocarbamate staining method or with a benzidine staining method after a preliminary HCl conc. exposure.

## 12. SERUM $\alpha_2$ (HS) GLYCOPROTEIN

Serum  $\alpha_2$ -HS-Glycoprotein tested in 18 chronic DLE patients showed large variations ranging between 44-192 L.U.

Levels over 130 L.U. were recorded in 4 patients, levels under 70 L.U. in other 4 patients.

# CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS (PRELIMINARY RESULTS).

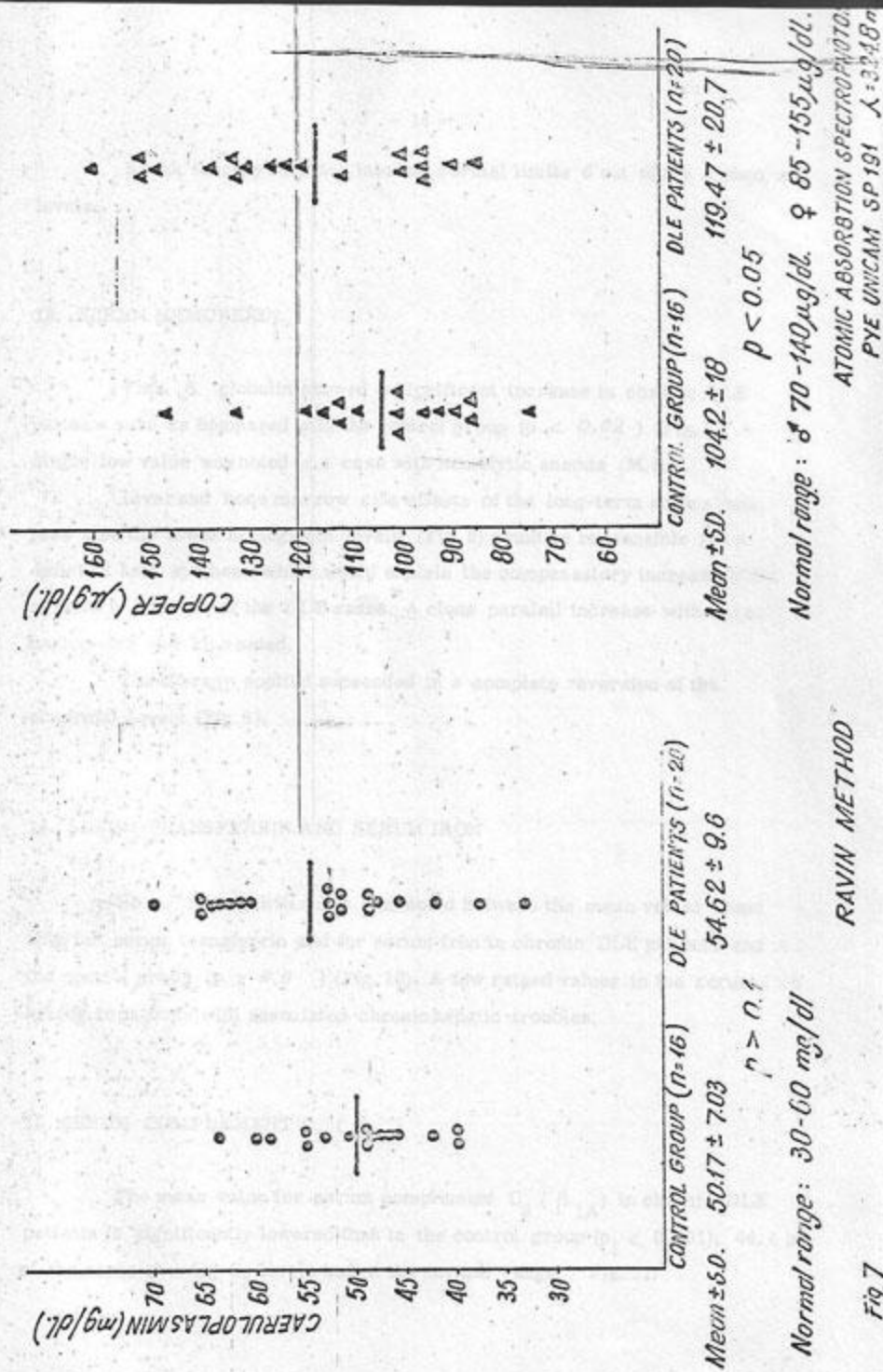


Fig. 7

Kozak therapy reverted into the normal limits 6 out of the 8 abnormal levels.

### 13. SERUM HEMOPEXIN

This  $\beta$  globulin showed a significant increase in chronic DLE patients sera as compared with the control group ( $p < 0.02$ ) (Fig. 8). A single low value was noted in a case with hemolytic anemia (M.S.).

Liver and bone marrow side effects of the long-term antimalaria (see also the lower hemoglobin levels (Fig. 9) could be responsible for a deficient hem synthesis which would explain the compensatory increase of its carrier in 44.4 pc of the DLE cases. A close parallel increase with serum haptoglobin was also noted.

The therapy applied succeeded in a complete reversion of the abnormal levels (Fig. 8).

### 14. SERUM TRANSFERRIN AND SERUM IRON

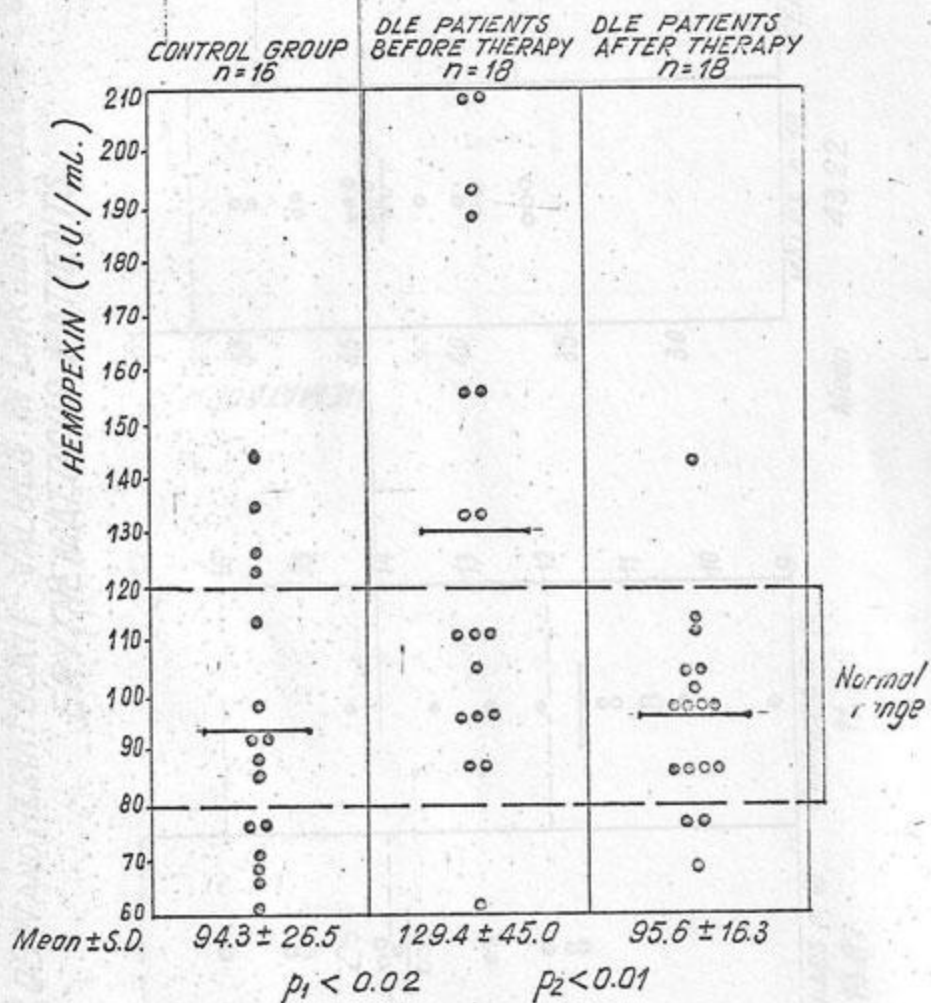
No significant difference was noted between the mean values found both for serum transferrin and for serum iron in chronic DLE patients and in the control group ( $p > 0.6$ ) (Fig. 10). A few raised values in the serum iron belong to patients with associated chronic hepatic troubles.

### 15. SERUM COMPLEMENT $C_3$ ( $\beta_{1A}$ )

The mean value for serum complement  $C_3$  ( $\beta_{1A}$ ) in chronic DLE patients is significantly lowered than in the control group ( $p_1 < 0.001$ ), 44.4 pc of the cases showing  $C_3$  levels under the normal range. Fig. 11.

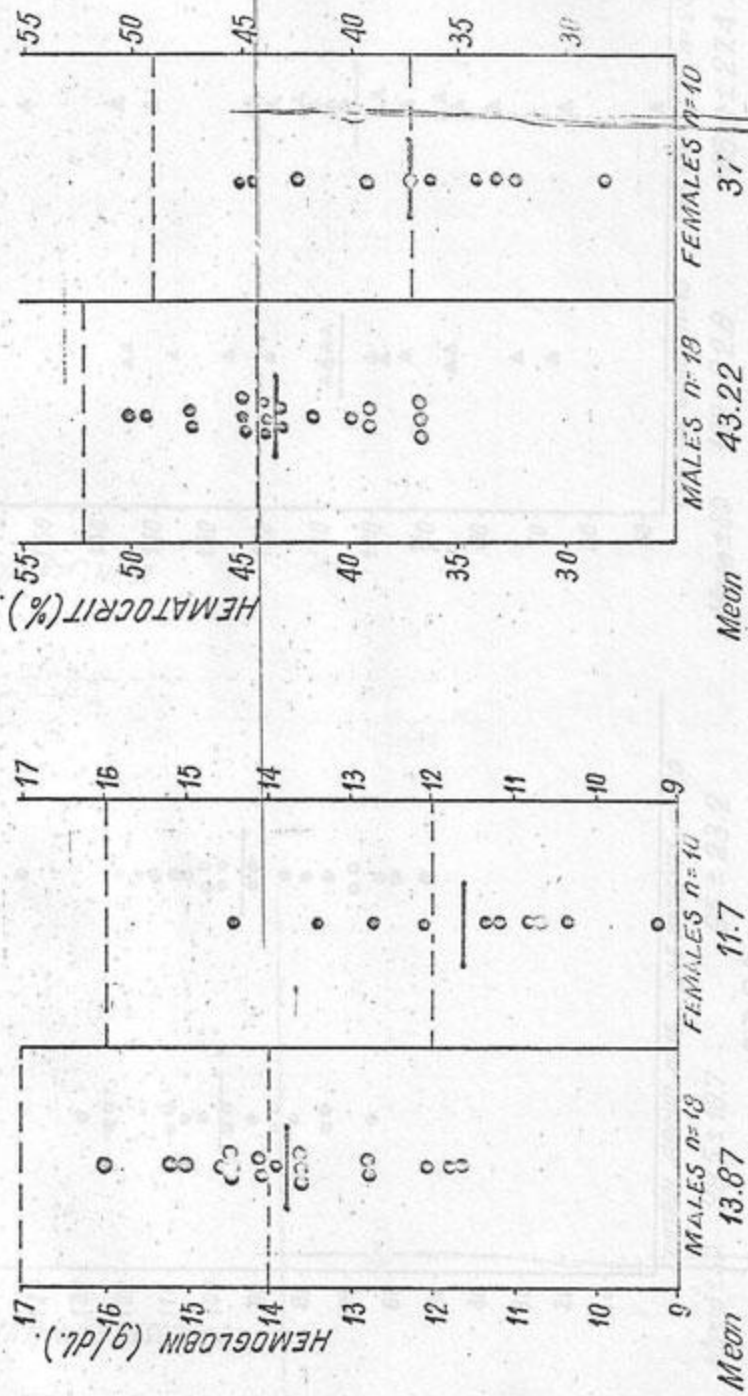


# SERUM HEMOPEXIN IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS BEFORE AND AFTER KOZAK TREATMENT. (PRELIMINARY RESULTS).



RADIAL IMMUNODIFFUSION

# HEMOGLOBIN AND HEMATOCRIT VALUES IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS.



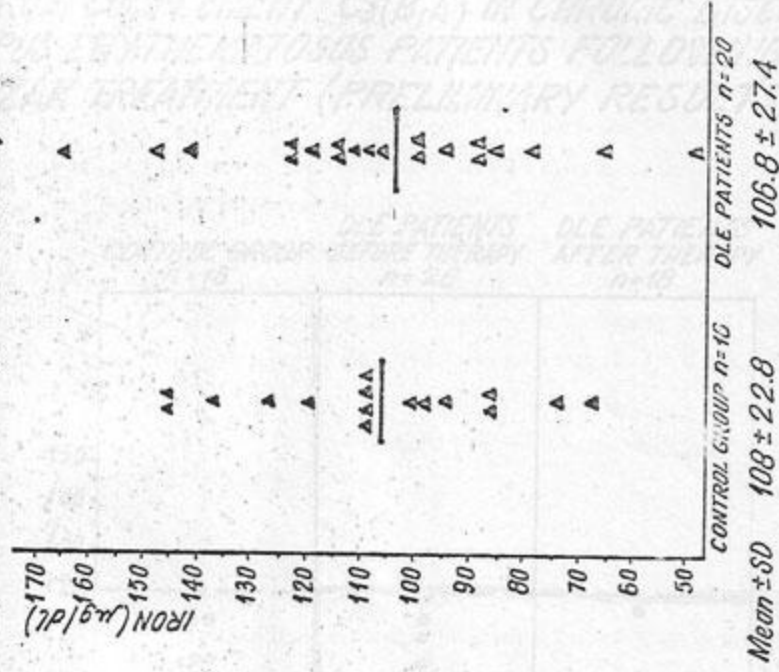
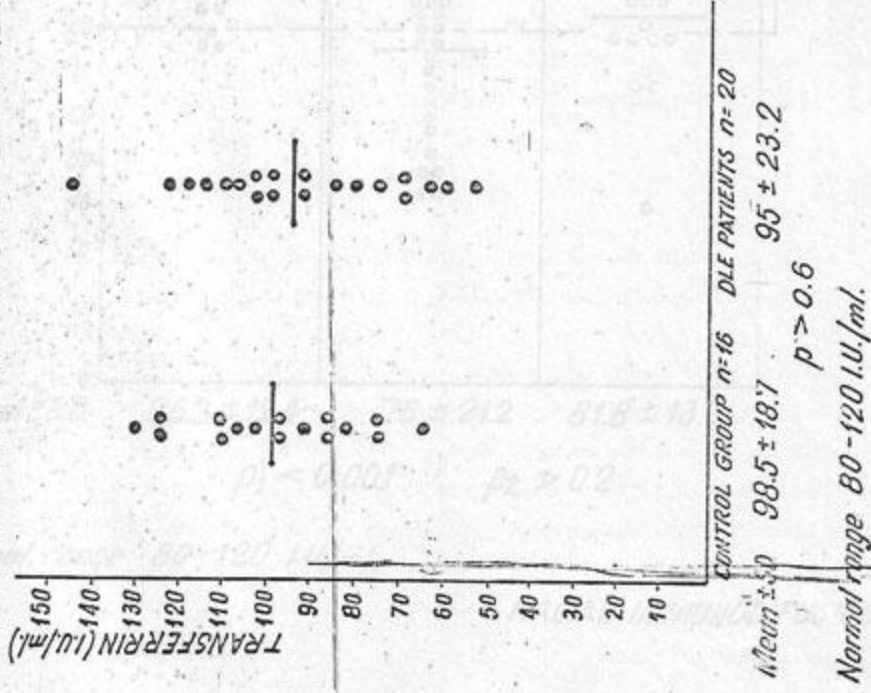
Normal range 14-17 g/dl

12-16 g/dl.

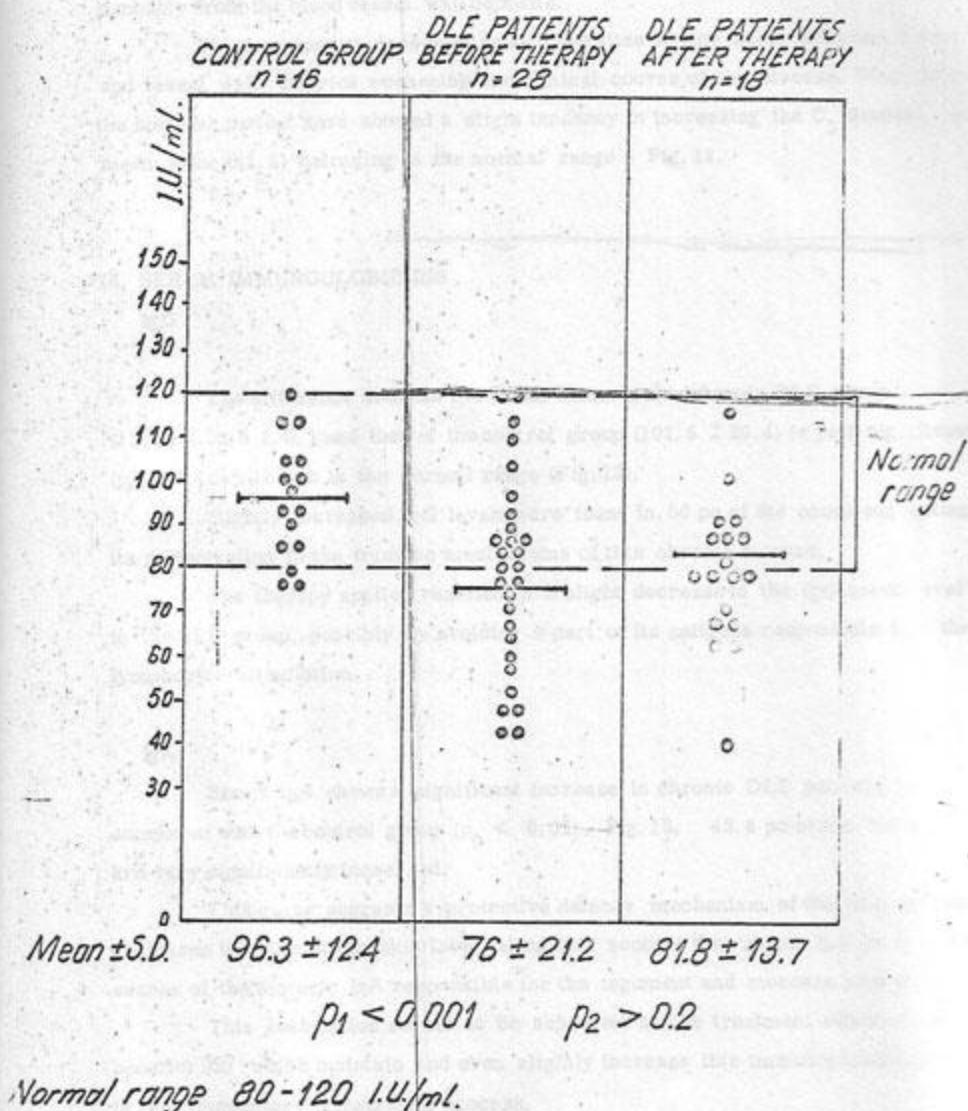
Normal range 44-52 %

37-49 %

# SERUM TRANSFERRIN AND SERUM IRON IN CONTROLS AND IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS. (PRELIMINARY RESULTS).



# SERUM COMPLEMENT C<sub>3</sub>(B<sub>1</sub>A) IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS FOLLOWING KOZAK TREATMENT (PRELIMINARY RESULTS)



RADIAL IMMUNODIFFUSION

This finding suggests an enhanced  $C_3$  consumption in the immune complexes from the damaged skin (linear deposits of immune complexes on the basal membrane) and probably from the blood vessel wall deposits.

The complement consumption at these sites brings about inherent tissue and vessel wall injuries worsening the clinical course of the disease. When leaving the hospital patient sera showed a slight tendency in increasing the  $C_3$  amount, the mean value (81.8) belonging to the normal range - Fig. 11.

## 16. SERUM IMMUNOGLOBULINS

### IgG

The difference between the mean value in the chronic DLE group ( $118.3 \pm 29.5$  I.U.) and that of the control group ( $101.5 \pm 20.4$ ) is just significant ( $p < 0.05$ ) although in the normal range (Fig. 12).

Slightly increased IgG levels were found in 50 pc of the cases suggesting its participation in the immune mechanisms of this chronic disease.

The therapy applied resulted in a slight decrease in the IgG mean level in the DLE group, possibly by avoiding a part of its antigens responsible for the lymphocyte stimulation.

### IgA

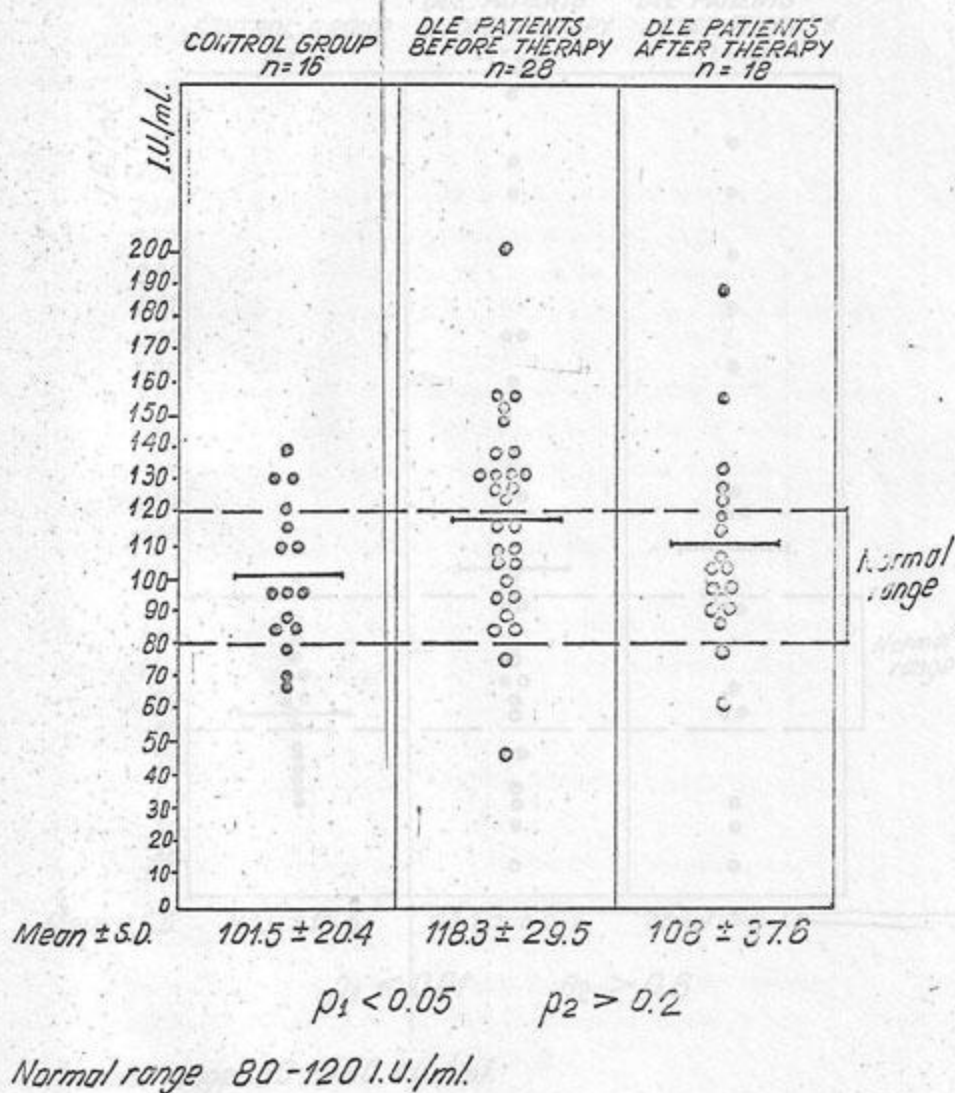
Serum IgA shows a significant increase in chronic DLE patients as compared with the control group ( $p < 0.01$ ). Fig. 13, 42.6 pc of the values are very significantly increased.

This raise suggests a protective defence mechanism of the host against the tissue injuries at the skin level taking into account that serum IgA is the main source of the dimeric IgA responsible for the tegument and mucosae protection.

This assumption seems to be supported by the treatment effects (clinically benefic) /2/ which maintain and even slightly increase this immunoglobulin necessary in the tegumentary cicatriceal process.

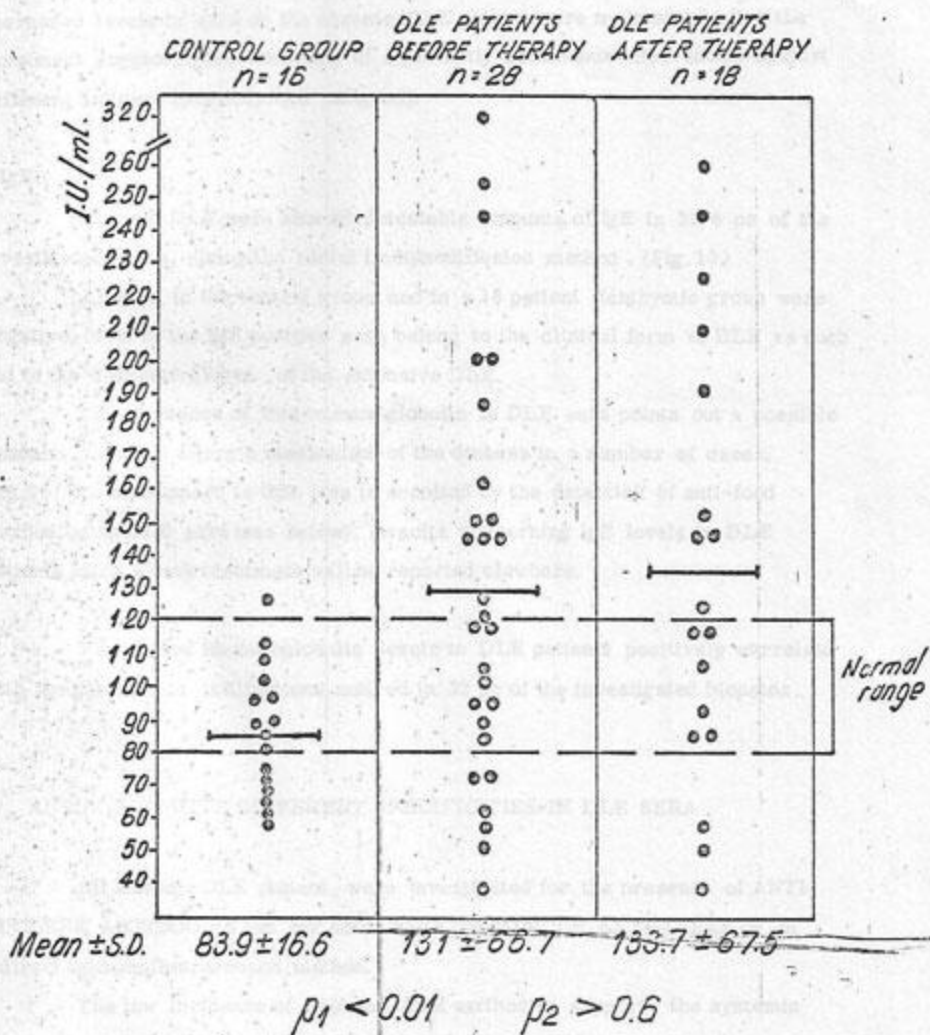


*SERUM IgG IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS  
PATIENTS FOLLOWING KOZAK TREATMENT.  
(PRELIMINARY RESULTS).*



RADIAL IMMUNODIFFUSION

# SERUM IgA IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS PATIENTS FOLLOWING KOZAK TREATMENT. (PRELIMINARY RESULTS).



RADIAL IMMUNODIFFUSION

### IgM

Serum IgM shows a similar behaviour to serum IgA. (Fig. 14) The increased levels in 42.8 of the chronic DLE patients are maintained after the treatment suggesting the necessity of a promptly continuous intervention against different antigens (possibly food antigens).

### IgE

Chronic DLE sera showed detectable amounts of IgE in 31.4 pc of the investigated cases, using the radial immunodiffusion method. (Fig. 15)

All sera in the control group and in a 16 patient ichthyotic group were negative. Most of the IgE positive sera belong to the clinical form of DLE as such and to the congestive form of the extensive DLE.

The presence of this immunoglobulin in DLE sera points out a possible basophil mediated allergic mechanism of the disease in a number of cases. (Fig. 16) Further support to this idea is supplied by the detection of anti-food antibodies in DLE sera (see below). Results concerning IgE levels in DLE patients after Kozak treatment will be reported elsewhere.

The raised immunoglobulin levels in DLE patients positively correlate with the plasmocyte infiltrations noticed in 32 pc of the investigated biopsies.

## 17. ANTIBODIES WITH DIFFERENT SPECIFICITIES IN DLE SERA

All chronic DLE patient were investigated for the presence of ANTI-NUCLEAR ANTIBODIES and for ANTI SMOOTH MUSCLE ANTIBODIES by an indirect immunofluorescence method.

The low incidence of ANA and ASM antibodies support the systemic non involvement in our patients (Table 1). Only the speckled pattern of anti-nuclear staining was noted in ANA positive cases.

**SERUM IgM IN CHRONIC DISCOID LUPUS ERYTHEMATOSUS  
PATIENTS FOLLOWING KOZAK TREATMENT.  
(PRELIMINARY RESULTS).**

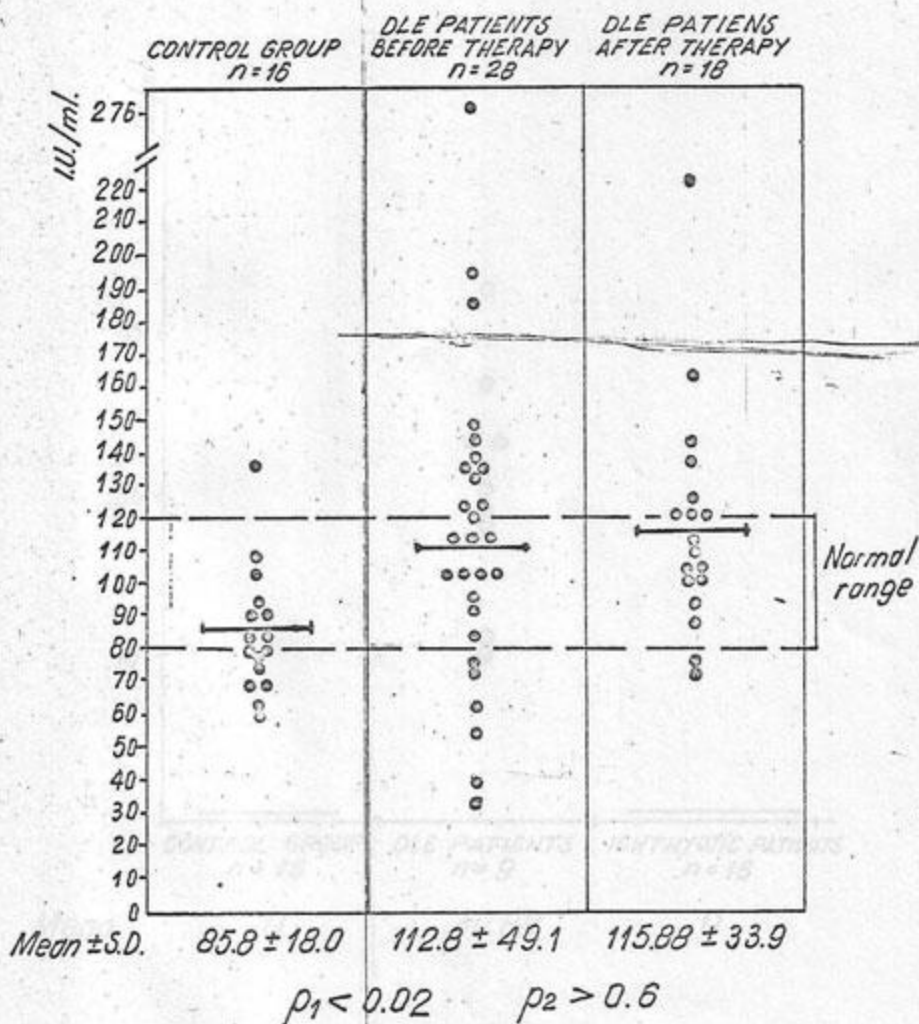
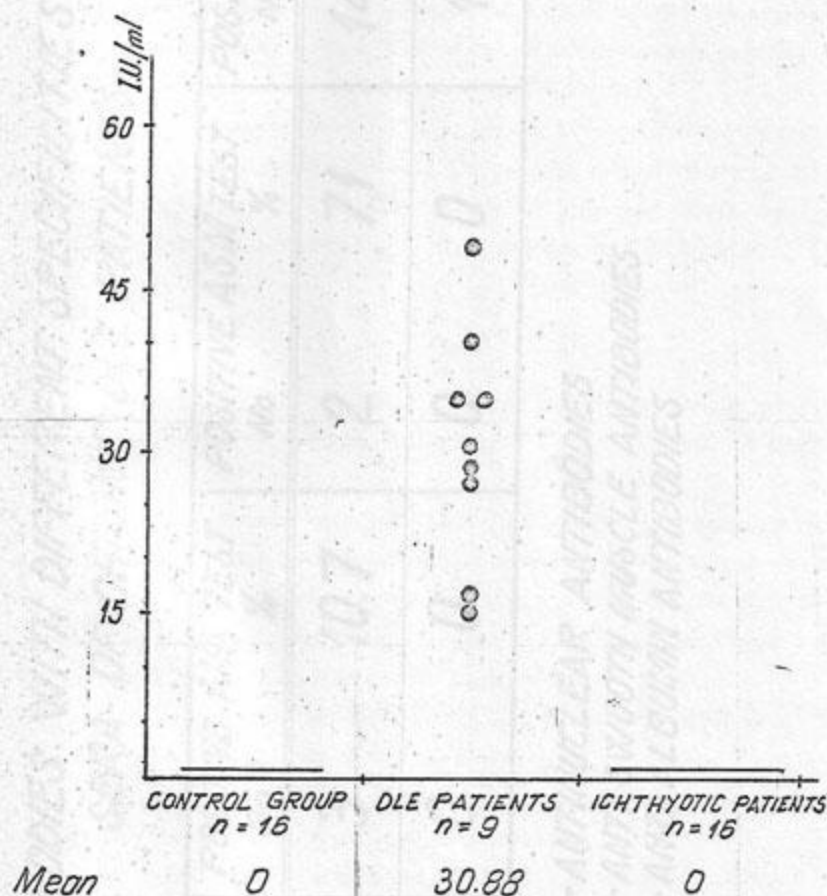


Fig. 14

# SERUM IgE IN CHRONIC DLE PATIENTS

## DECELABLE IgE IN 31.4 p.c. OF THE CASES



RADIAL IMMUNODIFFUSION  
BEHRING WERKE IgE L-PARTIGEN



TABLE 1.

# ANTIBODIES WITH DIFFERENT SPECIFICITIES IN SERA OF CHRONIC DLE PATIENTS.

No. TESTED	POSITIVE ANA TEST No	POSITIVE ANA TEST %	POSITIVE ASM TEST No	POSITIVE ASM TEST %	POSITIVE AAA TEST No	POSITIVE AAA TEST %
28 DLE PATIENTS	3	10.7	2	7.1	14	50
16 CONTROLS	0	0	0	0	1	6.2

ANA - ANTINUCLEAR ANTIBODIES  
 ASM - ANTI SMOOTH MUSCLE ANTIBODIES  
 AAA - ANTI ALBUMIN ANTIBODIES

#### ANTI-ALBUMIN ANTIBODIES (AAA)

AAA were present in 50 pc of the tested DLE sera (Table 1). The high incidence of these antibodies suggests certain changes in the serum albumin antigenical structure induced by long-term hepatotoxic agents. The previous antimalarial treatments would be involved in this respect as well as other different nocive compounds entering the organism via food.

The presence of the AAA may have an important role in the reduced albumin levels encountered in 43 pc of the patients, fact which brings about other negative side effects worsening the clinical state (see above). AAA serum levels are significantly reduced after Kozak treatment in DLE patients.

#### ANTI FOOD ANTIGENS

The relative high incidence of clinical recurrences in DLE patients who didn't observe the indicated diet prompted us to investigate the presence of anti-food antibodies in DLE sera.

We report here our results in 12 DLE and 6 control sera using purified egg albumin, total egg albumen and a lamb meat extract as antigens - Table 2.

A positive anti-total egg albumen test was found in 3 out of 12 DLE patients while the anti purified egg albumin test was negative. Antigenic differences should be responsible for this phenomenon.

A positive anti-lamb meat extract test was noted in 4 out of 12 DLE cases and in 1 out of 6 controls - Table 2.

The immunoglobulin class(es) involved in this reaction have not been yet determined but research on this topic is in progress.

Preliminary positive results with other food antigens provided further evidence for the importance of these recurrence inducers and possibly disease promoters.

Their continuous ingestion along with an inappropriate diet may provide circulating immune complexes which induce an enhanced platelet aggregation and/or basophil mediated allergic reactions. (Fig.16).

# INCIDENCE OF ANTI FOOD ANTIBODIES IN SERA OF CHRONIC DLE PATIENTS (PRELIMINARY RESULTS).

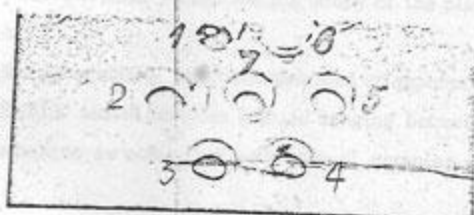
No. TESTED	POSITIVE ANTI PURIFIED EGG ALBUMIN TEST *	POSITIVE ANTI TOTAL EGG ALBUMEN TEST *	POSITIVE ANTI LAMB MEAT EXTRACT TEST **
12 DLE PATIENTS	0	3	4
6 CONTROLS	0	0	1

\*\* ANTIGEN CONCENTRATION : 30 mg./ml.

\*\* ANTIGEN CONCENTRATION : 15 mg./ml.

DETECTION BY OUCHTERLOMY METHOD

# ANTIBODIES AGAINST TOTAL EGG ALBUMEN IN CHRONIC DLE SERA



1. CONTROL SERUM
2. D.C. BEFORE THERAPY ( + + + )
3. D.C. AFTER THERAPY ( 0 )
4. C.R. BEFORE THERAPY ( + + )
5. C.R. AFTER THERAPY ( 0 )
6. CONTROL SERUM
7. EGG ALBUMEN ( 30 mg/ml. )

When leaving the hospital patients showed reduced or absent anti-food antibodies (seeslide) which positively correlated with a good clinical picture. /2/

#### 18. PLATELET AGREGATION

The low platelet count encountered in 85.7 pc of the DLE patients (values under  $120,000/\text{mm}^3$ ) inspired the study of the platelet aggregability in 12 DLE cases.

A high aggregability was demonstrated at appropriate doses of ADP or collagen in all DLE tested samples (values ranging between 40 and 65 pc of the absorbance reduction as compared with normal samples - 20 pc absorbance reduction).

This finding suggested the presence of a disseminated intravascular coagulation pattern continuously supplied by the immune complexes including food antigens or radicals which enter the organism via food. The albumin-anti albumin antibody complexes could also be responsible for the maintenance of this abnormal aggregability with negative clinical effects /2/. Kozak therapy reverted to the normal limits 10 out of the 12 abnormal values.

#### 19. THE CLOT RETRACTION TIME

The clot retraction time was significantly shorter in all 12 DLE cases than in controls. This fact copes with the lowered hematocrit values in DLE patients (Fig. 9) and support the pattern of an accelerated coagulation.

#### 20. FIBRINOGEN/FIBRINE SPLITTING PRODUCTS (FSP)

The FSP determined with the Staphiloclumping reagent showed a significant increase in the DLE samples.



All values were higher than  $10 \mu\text{g/ml}$  (range  $15-45 \mu\text{g/ml}$ ) demonstrating an enhanced fibrinolytic process as a reaction to the increased aggregability (Fig. 16).

The high plasminic activity involved in this process is counteracted by the raised levels of the  $\alpha_1$ -trypsin inhibitor and  $\alpha_2$ macroglobulin, the main antiplasminic factors (see above).

As the FSP are known to inhibit the reversible platelet aggregation it results that the  $\alpha_1$  trypsin inhibitor and the  $\alpha_2$  macroglobulin exert an indirect favourising effect upon the platelet aggregation.

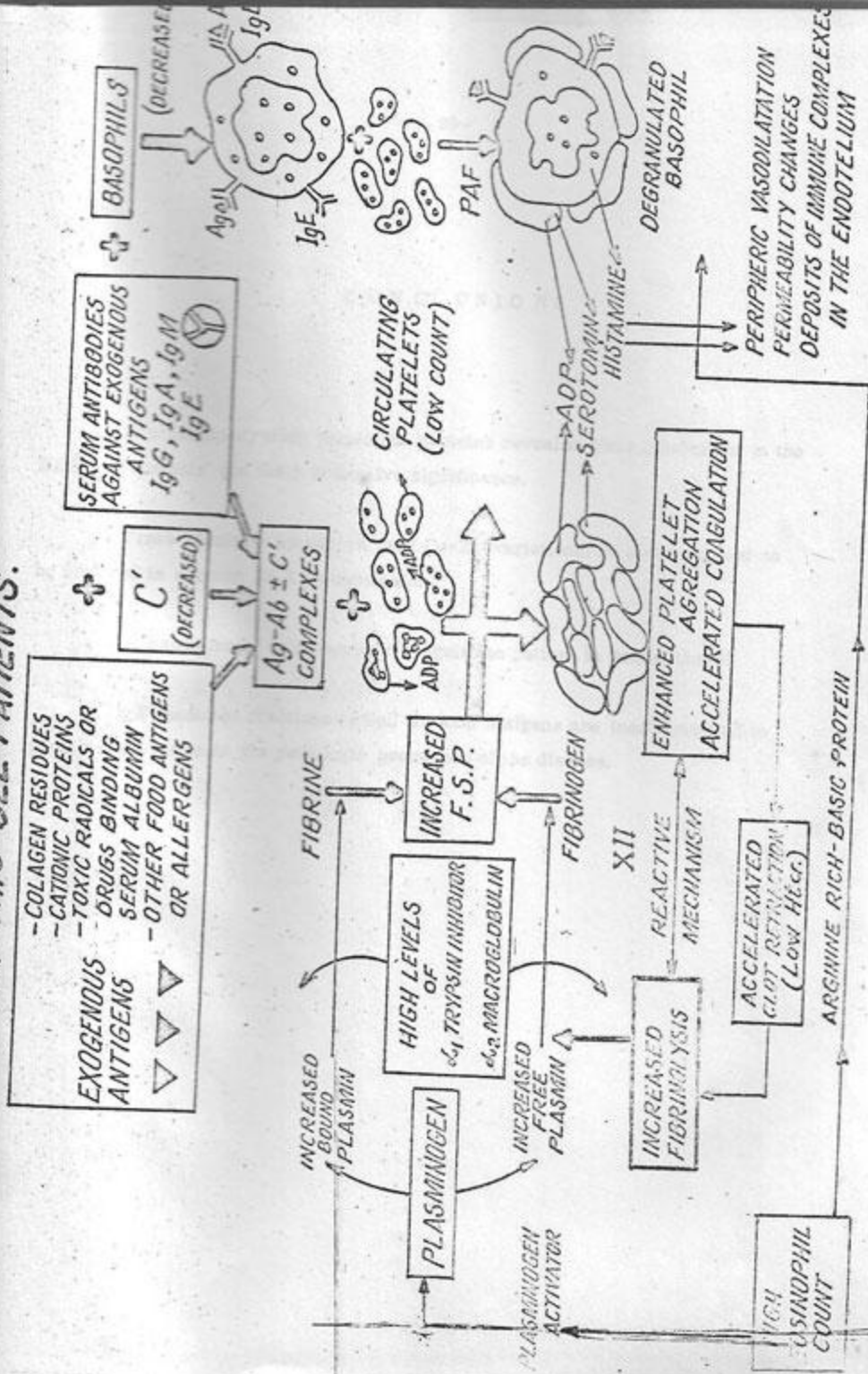
21. THE HIGH EOSINOPHILIC COUNT encountered in 32 pc of DLE patients could be responsible for a higher plasminogen activation necessary in the fibrinolytic mechanism.

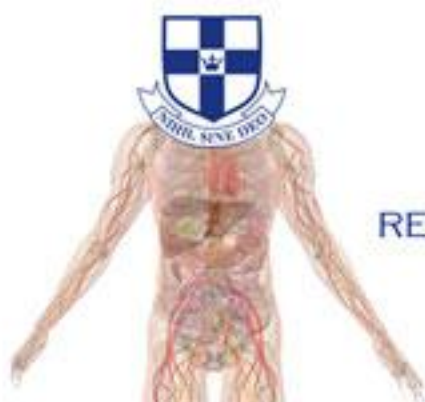
On the other hand eosinophils could neutralize the histamine - serotonin discharges in the damaged skin areas through their arginine-rich basic protein (Fig. 16),

22. BASOPHILS were absent in all blood smears investigated suggesting an enhanced participation in the peripheral inflammatory focuses.

A basophil mediated allergic mechanism already assumed by others could find further support in our chronic DLE patients where decelable IgE announs and an increased platelet aggregability have been demonstrated. (Fig. 16).

# CHRONIC DLE PATIENTS.





**CLINICA DR. KOZAK**  
RECONSTITUIM SANATATEA  
DIN INTERIORUL DVS..

## **CONTACT**

**Clinica Bucuresti**

**Str. Plantelor Nr.52A, Sector 2  
Bucuresti**

**Telefon (+4) 021 320 3002**

**Urgente (+4) 0740 093 333**

**Email: [contact@kozak-dermato.ro](mailto:contact@kozak-dermato.ro)**

**WEB: [www.kozak-dermato.ro](http://www.kozak-dermato.ro)**