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**The Application of Steffen's Antiglobulin Consumption-Test  
for the Detection of Anti-Organ-Antibodies**

Zastosowanie odczynu Steffena konsumpcji surowicy antyglobulinowej  
do wykrywania przeciwciał antytkanekowych

Применение реакции потребления антиглобулиновой сыворотки Стеффена  
для определения антитканевых аутоантител

In the last years autoantibodies have been divided into those which have etiological meaning for the disease and those without any pathogenic properties which are only witnesses of the immunologic process in the given organ. It should be mentioned that it is not the antibody alone but an antigen-antibody-complement complex that exercises this pathogenic activity (1).

Autoantibodies can be directed against organs and tissues, or against substructural fractions such as mitochondria, microsoms, nuclear antigens. In the same organism precipitating, agglutinating and complement fixing antibodies can be found by various serological methods (2).

To detect tissue antibodies the antiglobulin consumption test described by Steffen (3, 4) was used. Circulating, mostly incomplete antibodies were found by this method, using absorption with liophylized tissue or tissue homogenats. Antibodies present in the serum are specifically bound by the tissue antigens. After several washings of the residue with physiological saline, antiglobulin of known titer is added to the complex. The antigammaglobulin of the serum combines with the antigen-antibody-complex. After centrifugation the antiglobulinserum (supernatant) is again titrated. A drop in the titer constitutes an indirect evidence of the presence of antibodies in the examined serum. By the consumption test global antibodies to antigens of the used tissue are demonstrated (parietal, microsomal, mitochondrial). The aim of our examinations was to search for autoantibodies in sera of patients with Lupus erythematodus. As controls, sera of a group of patients with various diseases and a group of healthy persons were examined under the same conditions.

## MATERIAL OF PATIENTS

The patients were hospitalized and clinical observations were performed in the L. Rydygier Municipal Hospital in Wrocław. 20 patients with lupus erythematosus were subjected to manifold examinations, single examinations having been carried out with 3 patients only. In this group 19 patients had antinuclear antibodies, one diagnosis was made by skin biopsy performed two years ago in the Institute of Rheumatology in Warszawa. The antinuclear antibodies were determined by the immunofluorescence, the Latex-test (Hyland) and the Bentonit test for DNA.

Among 20 patients with different diseases we examined 5 patients with myeloma multiplex, 7 patients with thyroid gland diseases (6 with adenoma, 1 with subacute thyroiditis), 2 cases of post-alcohol liver cirrhosis, 4 patients with rheumatoid disorders, one case of chronic nephritis and one with infarctum myocardiæ. Out of 10 healthy persons, 7 were blood donors, 3 were hospitalized with neurosis. One person of the latter group was found to have an inactive duodenal ulcer.

## METHODS

In our experiments we used stroma of thyroid, kidney, adrenals, heart muscle and skeletal muscle cells. These organs were taken a few hours after death (4–6 hours) from healthy persons who had lost their lives in accidents.

The tissues were cut in small pieces and washed several times with physiological saline of pH 7.2 till no traces of blood were visible. After homogenization in a glass homogenizer for 5 minutes, the homogenate was washed as described above and liophilized (thanks to the kindness of the Blood Donor Station in Lublin). For absorption purposes 5–10 mg of the liophilized tissue was used for 4 ml of serum diluted 1:2. After 2 hours of incubation at 37°C the sera were centrifuged and washed 10-fold with cold saline. The antiglobulin serum was added to the residue, shaken for 2–3 minutes and then centrifuged. The absorbed antiglobulin serum was again titrated with 10% erythrocyte solution of ORh + blood group and coated with serum anti D (1 drop of the diluted serum + 1 drop of the appropriate erythrocyte solution). The drop of the titer of 2 test tubes was determined as weakly positive, the drop of 3 test tubes as moderately positive, the difference of 4 and more tubes as strongly positive.

## RESULTS

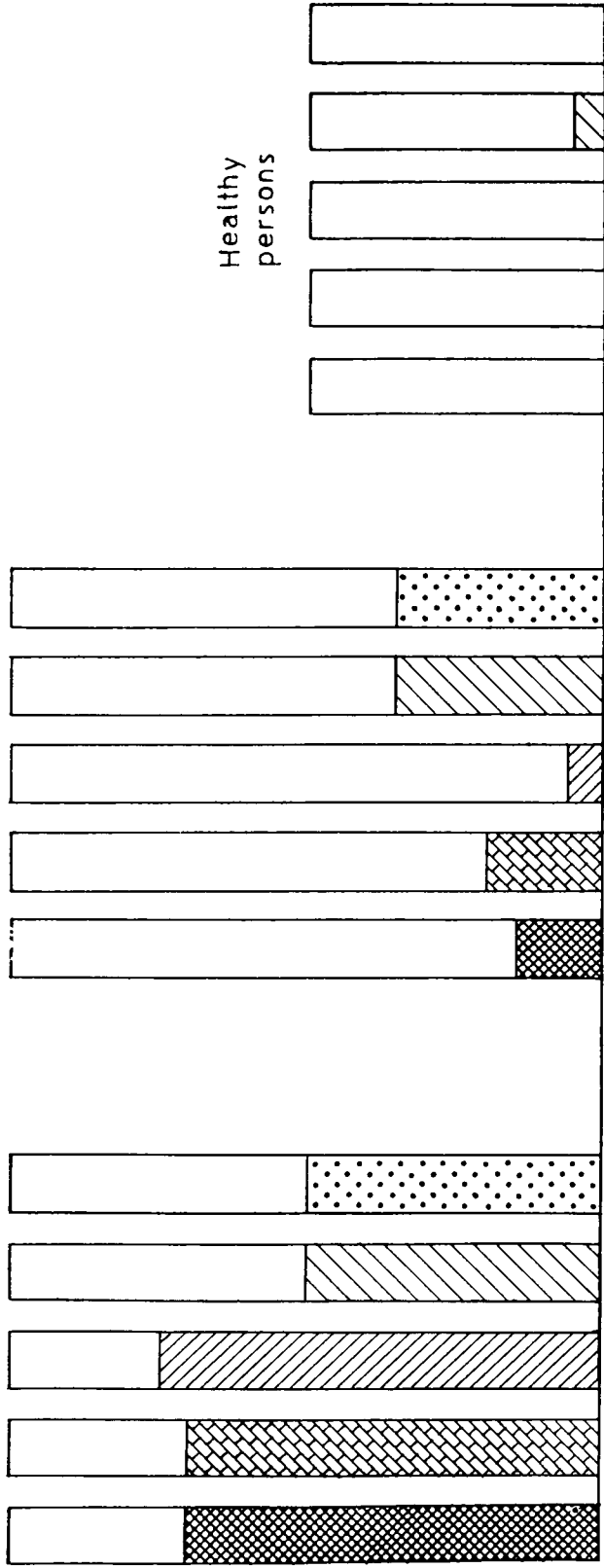
The results of our investigations are collected in fig. 1. In group I (*Lupus erythematosus*) positive results are in preponderance. 13 patients were positive with skeletal, 14 with heart muscle, 15 with kidney, 8 with adrenals and 8 with thyroid tissue. The results were mostly strongly positive, especially with the heart tissue, skeletal muscles and kidneys.

We want to stress the negative results with every tissue in sera from patients with myeloma multiplex in group II (various diseases) despite the marked hypergammaglobulinaemia. Three patients with rheumatoid disease showed a strong positivity with skeletal muscle; one patient with myocardial infarction (the serum was taken at the second week's end of

Various diseases

Lupus erythematosus

Healthy persons



- skeletal muscle
- heart muscle
- kidney
- adrenals
- thyroid

the disease) and done with rheumatoid disease were strongly positive; 2 with rheumatoid disease weakly positive with heart muscle.

Weakly positive was the result with kidney tissue of a twelve-year-old boy with chronic glomerulonephritis which had begun in his fifth year.

A strongly positive test with thyroid tissue was noted in the serum of a patient with subacute thyroiditis, while the sera from patients with adenoma were weakly positive. Positive and weakly positive with adrenal tissue were also the results of 7 patients without any dysfunction of adrenals.

#### DISCUSSION

There are 3 problems to which we wanted to draw the reader's attention:

1. The correlation between the clinical picture and the results in the Steffen's antiglobulin consumption test.
2. The influence of immunosuppressive therapeutics on the serological results.
3. The usefulness of the test in the appreciation of the clinical state and therapeutic results in prognosis.

These three problems may be treated as secondary to the general one: whether or not and to what extent the test is specific. We would like to show the correlation between the clinical state and the results in Steffen's test on the example of one organ: the kidneys. In every patient with Lupus erythematosus very accurate examinations of the kidneys' activity were performed several times. Eleven of them showed continuously *lupus nephritis*, 4 transient kidney disorders, 5 had no trace of clinical alteration of the kidneys. One person of the last group had a strongly positive Steffen test; after one year of immunosuppressive treatment, there was a drop in titer and the test became weakly positive. The strong positivity with skeletal muscle, thyroid and adrenals became quite negative. After 2 years we found a defect in the filtration of the glomeruli in the endogenous creatinine clearance.

In the group of patients with transient disorders in the kidneys, 2 sera were positive and 2 weakly positive by absorption with kidney tissue. Out of 11 patients in group III with constant disorders in the kidneys, 10 had positive results, 4 of which were strongly positive, 3 positive and 3 weakly positive. The last three were analysed during uremia. One patient, despite clinical symptoms of lupoid kidney inflammation, had a negative immunological result with all 5 tissues. Perhaps the autoantibodies were bound by the tissue *in vivo* (5). The application of immunosuppressive drugs caused a drop in the titer in Steffen's test with 88% of the patients.

In the light of our experience, mostly in connection with manifold examinations of sera of the same patients and in confrontation with other investigations and the clinical picture, we can say that the Steffen's anti-globulin consumption test may be of help in the evaluation of the degree of the inflammation process and also in prognosis.

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#### STRESZCZENIE

Celem pracy było oznaczenie autoprzeciwciał antytkankowych w surowicach chorych z toczeniem trzewnym. Kontrolną grupę stanowiły surowice chorych z różnymi innymi schorzeniami oraz surowice grupy osób zdrowych, badane w tych samych warunkach.

Materiał badany stanowiło 20 chorych z toczeniem trzewnym i 20 chorych z innymi schorzeniami, hospitalizowanych w Szpitalu Miejskim im. Rydygiera we Wrocławiu, i 10 dawców krwi.

Metodą użytą do wykazania przeciwciał był odczyn konsumpcji surowicy antyglobulinowej Steffena. Tkanki użyte do adsorpcji: tarczyca, nerka, nadnercza, mięsień sercowy i mięśnie gładkie uzyskano bezpośrednio po śmierci osób o grupie krwi 0. Tkanekę liofilizowano i użyto w dawce wymiareczkowanej (1—2,5 mg/ml) do adsorpcji. Czterokrotne obniżenie miana surowicy antyglobulinowej uważano za odczyn dodatni. Wyniki ujęte są w ryc. 1.

W grupie chorych z toczeniem 13 pacjentów wykazało przeciwciała przeciw tkance mięśnia gładkiego, 14 — przeciw tkance mięśnia sercowego, 15 — przeciw nerce, 8 — przeciw nadnerczom i tarczycy. Najwyższe miana stwierdzono po adsorpcji mięśniem sercowym i gładkim.

W innych schorzeniach pozytywne odczyny stwierdzano rzadko i w dużo niższych mianach. Wykazano zbieżność obrazu klinicznego i odczynów serologicznych oraz wpływ leczenia immunosupresyjnego na wyniki badań.

Odczyn Steffena okazał się przydatny w klinicznej ocenie stanu choroby i w ocenie wyników leczenia.

## РЕЗЮМЕ

Целью работы было определение антитканевых аутоантител в сыворотке крови больных *Lupus erythematosus*. Контрольную группу составляли сыворотки крови больных другими болезнями и сыворотки крови здоровых людей, исследованные в тех же самых условиях.

Экспериментальный материал — это 20 человек, больных *Lupus erythematosus*, 20 человек, больных другими болезнями (находящихся в Городской больнице им. Рыдыгера во Вроцлаве), а также 10 доноров.

Для определения антител был использован метод реакции потребления антиглобулиновой сыворотки Стаффена. Ткани, которые применялись в абсорбции (щитовидная железа, почка, надпочечная железа, сердечная мышца и гладкая мышца), брались у людей с нулевой группой крови непосредственно после их смерти. Ткани лиофилизировались и в абсорбции применялись в соответственно вытитрованной дозе (1—2 мг/мл). Четырехкратное понижение титра антиглобулиновой сыворотки принималось за положительную реакцию.

Результаты приведены в рис. 1.

У 13 из 20 пациентов, больных *Lupus erythematosus*, были обнаружены антитела против ткани гладкой мышцы, у 15 — против ткани печени, у 14 — против ткани сердечной мышцы, у 8 — против тканей надпочечников и щитовидной железы. Самые высокие титры были установлены после абсорбции сердечной и гладкой мышцами.

У больных другими болезнями положительные реакции обнаруживались редко и в более низких титрах. Доказано сходство клинической картины и серологических реакций, а также влияние иммуносупрессионного лечения на результаты исследований.

Реакция Стеффена оказалась пригодной для клинической оценки состояния болезни и для оценки результатов лечения.