constituants, ceux-ci sont issus de cette solution de continuité et sont alors rassemblés sous forme de nouvelles bandes.

En conclusion. L'obtention par élution de fractions parfaitement homogènes après électrophorèse en gel d'amidon, est extrêmement difficile et doit être soumise comme contrôle de pureté à l'analyse immunoélectrophorétique, capable par sa sensibilité de révèler éventuellement l'hétérogénéité de la fraction envisagée.

J. M. FINE et J. LOEB

Centre National de Transfusion sanguine, Paris, le 3 novembre 1958.

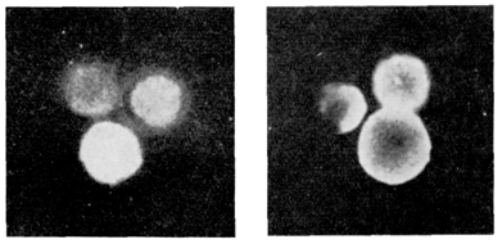
Summary

Human plasma components, separated by starch gel electrophoresis, are eluted and concentrated by lyophilisation. Immuno-electrophoretic analysis shows that certain of these fractions are immunologically heterogenous.

The authors point out that this immunological criterium should be used before employing an eluted fraction, even if it appears electrophoretically homogenous.

The Demonstration of D (Rh) Antigen in Human Leukocytes

Observations on the presence of D (Rh) antigen in human leukocytes are scarce and contradictory[1,2]. Results described in this communication indicate that D antigen may be detected in white cells from Rh-positive persons by means of the fluorescent antibody technique first described by COONS and KAPLAN[3].



A *B*

The staining technique consists of three steps: (1) incubation of leukocytes with anti-D serum; (2) exposure of sensitized white cells to chicken anti-human globulin serum, and (3) staining with anti-chicken globulin fluorescein conjugate (kindly prepared by Dr. A. PRINCE). The leukocytes from Rh-positive and Rh-negative persons were investigated. The controls were as follows: (a) washed cells, examined for autofluorescence; (b) leukocytes directly exposed to fluorescein conjugate; (c) sensitized white cells stained with fluorescein conjugate; (d) leukocytes treated with chicken anti-human globulin serum and incubated with conjugate, and (e) white cells from Rh-positive persons incubated in normal human serum which does not contain anti-D antibody. A Reichert fluorescent microscope equipped with a high pressure mercury vapor lamp and dark field condenser was used. The photographs were taken on Agfa Fluorapid Film which is particulary sensitive to yellow-green light.

Typical results are shown in the Figure in *A* the white cells are seen in ordinary dark field, and in *B* with ultraviolet light. Controls are equally visible in ordinary dark field, but did not fluorescence yellow-green. A weak bluish autofluorescence was sometimes visible. Positive results are only obtained with leukocytes from Rh-positive persons. The full report will be published elsewhere.

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B. D. JANKOVIĆ and T. L. LINCOLN

Microbiological Institute, Belgrade University School of Pharmacy, Belgrade (Yugoslavia), September 30, 1958.

Zusammenfassung

Mit indirekter Fluoreszenz-Antikörper-Methode wurde nachgewiesen, dass Leukozyten Rh-positiver Versuchspersonen D-Antigen enthalten.

[1] B. W. GURNER and R. R. A. COOMBS, Vox Sang. *3*, 13 (1958).
[2] J. DAUSSET, J. COLOMBANI, and J. EVELIN, Vox Sang. *3*, 266 (1958).
[3] A. H. COONS and M. H. KAPLAN, J. exp. Med. *91*, 1 (1950).

Immunology of Toxemias of Pregnancy II. Immunological Reactivity in Eclamptic Conditions

In the first part of this study we have pointed out the presence of autoantibodies in the sera of pregnant women suffering from preeclampsia. In this part we are following immunological reactivity in this disease.

Materials and Method. A group of 86 women whose pregnancy had lasted from 2 to 8 months were each given a single antigenic stimulus by injecting subcutaneously 0·5 ml Brucella bacterine of Nr. 2 density of the McFarland scale. The immunization had been preceded by control tests for antibody to Brucella, with uniformly negative results. The response by antibody was assessed thrice: on the 7th, the 14th and the 21st days following the impulse. Evaluation of immunological reactivity was based solely on findings of incomplete antibodies. In cases where their titre did not exceed the titre of agglutinins, it was presumed that both titres were identical. The method employed had been described in detail in previous papers[1-3].

Only those women whose history was free of brucellosis, allergic diseases, acute rheumatic fever and acute diffuse glomerulonephritis were selected for the research. Simultaneously with the antigenic impulse, they were subjected to a thorough medical and gynaecological investigation; in none of them was the result pathological.

After parturition the women were divided, according to their course of pregnancy following the immunization, into a group with pathological (preeclamptic) course of pregnancy (19 cases), and into a control group (67 cases). Symptoms adopted for the determination of toxemia of

[1] V. WAGNER, V. REJHOLEC, and V. MALÝ, Ann. rheum. Dis. *14*, 243 (1955).
[2] H. PADOVCOVÁ, V. REJHOLEC, V. MALÝ, F. SUDA, and V. WAGNER, Ann. Paediatr. *187*, 351 (1956).
[3] V. REJHOLEC, V. FENCL, V. WAGNER, M. REPIČ-ŠLECHTA, and E. HALLEROVÁ, Minerva nefrolog. *2*, 3 (1955).

pregnancy were: Finding of albumin in the urine, increase in blood pressure above 150/100 mg Hg, swellings.

Results. Statistical evaluations did not reveal any association between the age of the women, or between the advance of pregnancy, on the one side and elevation of the antibody titre, or the incidence of toxemia on the other. This made it possible to treat each of the groups as a whole while comparing their immunological reactivity.

Fig. 1

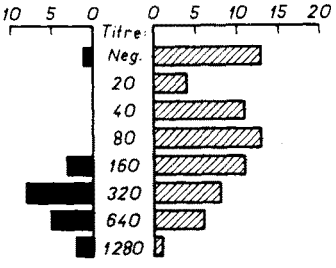


Fig. 2

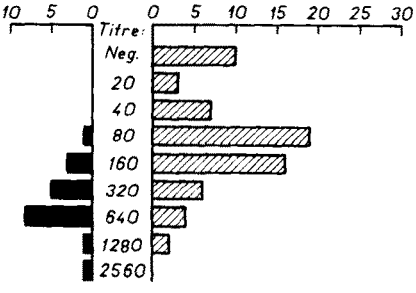
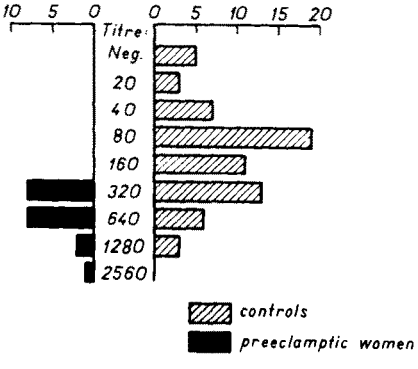


Fig. 3



It was found that already on the 7th day after the antigenic impulse the titres of incomplete antibody to Brucella antigen were statistically significantly higher in the preeclamptic group than in the control group ($P = 0{\cdot}05$). This difference still increased on the 14th and the 21st day and became higly significant ($P = 0{\cdot}01$). Figure 1 and 2 show the result of the titration of incomplete antibody on the 14th and the 21st days following immunization, and Figure 3 brings a comparison of all individual top titre levels irrespective of the time elapsed since immunization. All these graphs, but especially clearly the last one, bring to light the elevation of incomplete antibody titres in the group of preeclamptic women in comparison with the controls.

Discussion. The increase in the ability to produce incomplete antibody has already repeatedly been demonstrated for acute rheumatic fever[1,2], and acute glomerulonephritis[3]. The observations which are reported here suggest that preeclamptic conditions may also be classed with the diseases characterized by an increased immunological reactivity. On the other hand, our results do not reveal the mechanism by which hyperreactivity predisposes to pathological developments. It is true that in the group of preeclamptic women there was not a single case in which incomplete antibody to Brucella antigen was not present in a relatively high titre. Inversely, there were several pregnant women in whom signs of preeclampsia were lacking but who also reacted to the Brucella stimulus with a significant titre. Thus, it may well be that hyperreactivity is only one of the essential preconditions for the genesis of toxemia of pregnancy. Some further factor or complex of factors may be involved in this pathogenesis. For acute rheumatic fever and for acute glomerulonephritis, the etiological role of streptococcus pyogenes has been established: no similar agent has so far been detected for preeclampsia. Possible complicity of the foetus has been suggested by a number of workers but as yet not clearly defined. The auto-immunization processes described may well be conditioned primarily by hyperreactivity, but one should not leave out of account further possible factors, such as the easiness with which the antigenic structure of tissues may change, a certain antigenic affinity of different organs, as well as external influences which may also come into play in antigenic changes in the organism.

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Résumé

Après une seule injection d'un vaccin brucellique on trouve chez les femmes atteintes d'éclampsie une production d'anticorps incomplets significativement plus élevée que chez les femmes dont la grossesse a été normale.

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Maintenance of the Ability of Cells Cultivated *in vitro* to Commence Formation of Antibodies

It has been demonstrated in a number of papers[1] that mesenchymal cells removed from immunized donors continue to produce antibodies in tissue cultures. It is, however, still doubtful whether production of antibodies can be demonstrated in tissue cultures if the tissue is explanted shortly after injection of the antigen, i.e. up to 48 h, during the inductive phase. The majority of experiments in which antigen was added to an explantate of tissue from a non-immunized donor gave negative results[2]. Recently a few positive results have been reported[3].

In experiments performed during recent years, it was shown in our laboratory[4] that spleen tissue explanted

[1] A. FAGRAEUS, Acta med. scand. *130*, Suppl. 204 (1948). – G. J. THORBECKE and F. J. KEUNING, J. Immunol. *70*, 129 (1953); J. infect. Dis. *98*, 157 (1956). – A. B. STAVITSKY, J. Immunol. *75*, 214 (1955). – D. STEINER and H. ANKER, Proc. nat. Acad. Sci., Wash. *42*, 580 (1956).

[2] R. C. PARKER, Science *85*, 292 (1937). – A. J. SALLE and W. A. MCOMIE, J. Immunol. *32*, 157 (1937). – D. STEINER and H. ANKER, Proc. nat. Acad. Sci., Wash. *42*, 580 (1956).

[3] W. HÖPKEN, Virchows Arch. *325*, 39 (1954). – K. M. STEVENS and J. M. MCKENNA, Nature *179*, 870 (1957); J. exp. Med. *107*, 537 (1958).

[4] J. ŠTERZL and M. RYCHLÍKOVÁ, Czechoslov. Microbiol. *2*, 334 (1957); Folia biol. *4*, 11 (1958).