

Fig. 2. Agrégation plaquettaire en présence de DEAE-Dextran. PRP témoin: 260000 plaquettes.

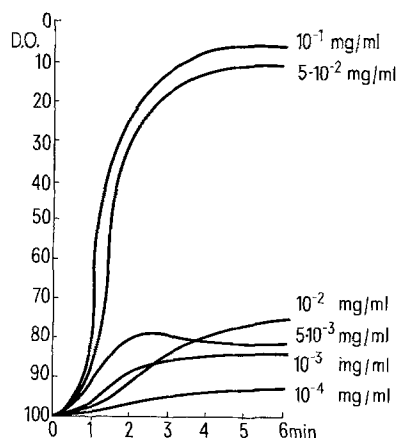


Fig. 3. Agrégation plaquettaire en présence de Polybrène. PRP témoin: 234000 plaquettes.

$10^{-1}$  mg/l. Remarquons d'ailleurs que les doses critiques ainsi trouvées correspondent à celles rapportées par d'autres auteurs<sup>8</sup>.

**Discussion-conclusion.** Les polybases telles que DEAE-Dextran et Polybrène diminuent la mobilité électrophorétique des plaquettes et induisent parallèlement une agrégation des plaquettes.

Il est probable que ces deux agents sont absorbés sur la surface plaquettaire. Ces constatations expérimentales

confirment alors la corrélation probable qui existe entre le phénomène d'agrégation des plaquettes et la charge superficielle de celles-ci.

Le mécanisme d'action de ces polybases reste encore à préciser. Cependant, il est probable qu'elles agissent par diminution des forces électriques facilitant ainsi l'établissement d'une « liaison » entre les plaquettes. L'étude des isothermes d'absorption de ces macromolécules à l'aide de suspensions de plaquettes lavées pourrait être envisagée.

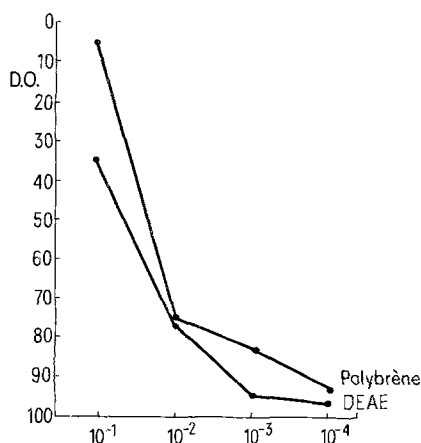


Fig. 4. Agrégation plaquettaire à la sixième min de contact.

**Summary.** The action of two polybases (polybren and DEAE Dextran) on the electrophoretic mobility of platelets is studied. These two substances induce a decrease of the mobility. An investigation of the aggregability with the help of a photometric test shows a correlation between the charge decrease and platelet aggregation.

A. LARCAN, F. STREIFF, J. F. STOLTZ,  
P. ALEXANDRE et A. NICOLAS

Groupe de Recherche Hémostaseologique,  
Centre Régional de Transfusion Sanguine et  
Service de Réanimation, CHU, Nancy 54 (France),  
15 mars 1972.

<sup>8</sup> T. PFLEIDERER et R. BROSSMER, *Thromb. Diath. haemorrh.* 18, 673 (1967).

## Human Red Cells Receptors and Immune Adherence Haemagglutination

The immune adherence haemagglutination (IAH) phenomenon consists in agglutination of human red cells (HRC) when exposed to an antigen-antibody complex, in presence of complement<sup>1-4</sup>.

In previous research, we found that not all the blood samples are suitable for the reaction, and reported individual differences (strong, weak and negative) independent of blood groups or storage of blood sample<sup>5</sup>. We showed that the type of reactivity is a fixed pro-

perty<sup>5,6</sup>, the negativity being presumably due to an autosomal recessive gene<sup>6</sup>. The behaviour of the HRC is not related either to type of antigen used in the reaction (soluble antigen, tissue culture cell extract, tissue culture monolayer or isolated tissue culture cells), or to the evolution of the IgM and IgG antibodies<sup>7-9</sup>.

In the present work, we were interested in investigating whether the individual differences among HRC, described by us in the IAH are caused by the presence or absence

of a surface receptor on the HRC. For this purpose, several blood samples have been selected according to their behaviour in the IAH (strong, weak, negative), and attempts have been made: a) to adsorb the IAH components (antigen-antibody complex and complement) comparatively on strong, weak and negative HRC; b) to remove the presumable receptor from the positive HRC and to use it as an inhibitor of the IAH; c) to immunize rabbits with HRC of different reactivity and to prepare specific antibodies to the presumable receptor.

a) In the first group of experiments, blood samples with different reactivity were incubated with the antigen-antibody complex (chick fibroblast extract-rabbit anti-chick fibroblast serum) in presence of complement as for a regular IAH test. After 30 min at room temperature, supernatants were removed by centrifugation and were used to perform again an IAH reaction by adding them to fresh untreated HRC, known to be strong positive in IAH. When added to the fresh strong positive HRC, supernatant removed from strong positive HRC generally gave negative results or very low titers; supernatants removed from weak HRC gave titers lower than before incubation; meanwhile supernatant removed from negative HRC allowed high titers of IAH. Similar results were obtained when ghosts prepared from blood samples with different reactivity (strong, weak, negative) were incubated with the antigen-antibody complex in presence of complement, and supernatants, removed for subsequent IAH with fresh untreated HRC known to be positive in IAH.

b) In order to remove the presumable receptor, trypsinization of different HRC was performed, and the HRC used further in IAH reactions. Strong positive HRC became negative for IAH, after trypsinization, meanwhile negative ones were not influenced by this treatment. Supernatants obtained by trypsinization of positive HRC, and added to an antigen-antibody complex in presence of complement, were able to inhibit the IAH with known positive HRC. Supernatants removed from trypsinized negative HRC did not inhibit the IAH.

c) Rabbits have been immunized, respectively, by a single inoculation with strong positive and negative HRC from different donors and with ghosts prepared from these HRC. Sera were withdrawn every day between 5-9 days after inoculation, so as to avoid formation of a large amount of anti species antibodies and to obtain, as far as possible, antibodies directed chiefly against the surface receptor. These sera were inactivated by heating at 56°C for 30 min; samples from every sera were adsorbed on HRC known as negative in the IAH, so as to remove the anti HRC antibodies. Haemagglutinating properties of nonadsorbed and adsorbed sera were checked comparatively with positive and negative HRC from different donors. Sera from rabbits immunized with positive HRC showed relatively high titers of haemagglutination with positive HRC and low titers with negative HRC; after adsorption on negative HRC, these sera lost

their ability to agglutinate negative HRC, but kept their agglutinins for positive ones. Sera from rabbits immunized with negative HRC had low titers of haemagglutination equal for both positive and negative HRC; after adsorption on negative HRC, their agglutinins decreased equally for positive and negative HRC.

Sera from rabbits immunized with ghosts prepared from positive and negative HRC gave results similar to those obtained with sera from rabbits immunized with fresh HRC. It is worth noting also that sera from rabbits immunized with positive HRC and adsorbed on negative HRC were able to agglutinate microscopical fragments obtained by supersonic vibration treatment of stroma prepared from positive HRC.

Our results lead to the conclusion that a surface receptor characterizes the HRC which are suitable for the IAH. This receptor, which can be removed by trypsin, seems to be abundant on strong positive HRC, while only small amounts are present on the weak HRC.

We do not know as yet whether this receptor has a biological role or whether it is only a genetic marker of the HRC<sup>10</sup>.

**Résumé.** Trois types d'hématies humaines sont décrites (fortes, faibles, négatives) selon leur réactivité dans l'adhérence sérologique (agglutination d'hématies humaines, exposées à un couple antigène-anticorps, en présence du complément). Ces différences individuelles sont dues à un récepteur dont la présence sur les hématies positives, est démontrée directement (adsorption des réactifs sur les hématies positives) et indirectement (perte de la réactivité par trypsinisation, inhibition de la réaction et préparation de sérums spécifiques anti-récepteur).

JEANNA SCHWARTZ, RUTH LEVY and  
NURITH VARDINON

Medical School, Department of Human Microbiology,  
Tel-Aviv University, Ramat-Aviv,  
Tel-Aviv (Israel), 9 February 1972.

<sup>1</sup> C. LEVADITI, Ann. Inst. Pasteur 15, 894 (1901).

<sup>2</sup> R. A. J. NELSON, Science 118, 733 (1953).

<sup>3</sup> C. LAMANNA, Bact. Rev. 1, 30 (1957).

<sup>4</sup> J. L. TURK, Immunology 1, 305 (1958).

<sup>5</sup> A. KLOPSTOCK, J. SCHWARTZ and N. ZIPKIS, Vox Sang. 8, 382 (1963).

<sup>6</sup> A. KLOPSTOCK, J. SCHWARTZ, Y. BLEIBERG, A. ADAM and A. SZENBERG, Vox Sang. 14, 177 (1965).

<sup>7</sup> J. SCHWARTZ, N. RUTMAN and N. VARDINON, Vox Sang. 14, 310 (1968).

<sup>8</sup> J. SCHWARTZ, N. RUTMAN, N. VARDINON and E. ROSENFELD, Int. Arch. Allergy 36, 204 (1969).

<sup>9</sup> J. SCHWARTZ and N. VARDINON, 4th Inst. Congress of Human Genetics (Paris), Excerpta Medica 233, 161 (1971).

<sup>10</sup> Acknowledgment. We thank Mr. I. OFEK and Mrs. NAVA RAZ for their competent technical assistance.

## Laser-Induced Excitation of Fluorescein Isothiocyanate in the Immunofluorescence

In recent years immunofluorescent procedures have been increasingly used in basic as well as in applied immunology. This was to be expected, since the immunofluorescence method is extremely sensitive. BARTELS<sup>1</sup> reported that quantities as small as 10<sup>-18</sup> g of fluorescein isothiocyanate (FITC), roughly corresponding to 1000 mole-

cules, could be detected by this method. So far only radioisotope tracers could be detected in such small concentrations.

At present it is not feasible to take full advantage of this method. There are still many facets which have not been solved. Let us mention only two of them: the effi-