from the granulocyte subfractions II and III by kaolin and eluted at alkaline pH.

At the present state of experimental evidence, it is not possible to reach a conclusion as to whether the granulocyte and plasma-clotting factors are identical. RAPAPORT and HJORT[8] found that rabbit neutrophils do not bind plasma-clotting factors. On the other hand, there is a certain possibility that some plasma-clotting factors may originate from granulocytes. The experiments with adsorption of procoagulant activity by kaolin from granulocyte subfractions and elution at alkaline pH provides further experimental evidence that factor XII (Hageman) may be localized in granulocytes. The specific procoagulant activities are much more concentrated in leucocytes than in plasma. For this reason, granulocytes, as well as platelets, may be considered as active centers in the formation of hemostatic plug.

It is of interest to note that procoagulant activity is mainly localized in the supernatant after 8200 *g* and in the sediment at 8200 *g*. The enzymes forming kinins were also found in the same fractions[9]. Since the work of MARGOLIS appeared[10], it is known that the formation of kinins may be initiated by the active Hageman factor. These facts could indicate the role of granulocyte factor XII for the generation of kinins in synovial fluid of patients with rheumatoid arthritis[11]. It has been suggested that the procoagulant activity of the leucocytes may be involved in the formation of fibrin in inflammatory exudates and in the intravascular clotting[12]. The present communication confirms this point of view.

Résumé. Les extraits granulocytaires racourcissent le temps de coagulation de plasmas déficients en facteurs VIII, IX et XII. Une activité paracoagulante particulièrement élevée se manifeste dans les subfractions de granulocytes contenant des lysosomes et d'autres structures cytoplasmatiques.

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The Positive Direct Coombs Test of Cephalothin-Treated Blood

MOLTHAN et al.[1] have recently reported that the antibiotic cephalothin, incubated in vitro with normal blood under suitable experimental conditions, produces a positive direct Coombs test; moreover, they observed that the antiglobulin test was slightly stronger when the blood of 4 azotemic patients was used instead of normal blood. According to the authors, the erythrocytes from patients with poor kidney function could be more sensitive to cephalothin than normal cells. We studied the in vitro action of cephalothin on the blood of 14 healthy subjects, 14 azotemic patients and 13 patients suffering from classical (portal) cirrhosis of the liver. Four volumes of blood, drawn with acid-citrate-dextrose, were incubated with one volume of cephalothin (Keflin Lilly) dissolved in saline at different concentrations (final concentration of the mixtures = 0.625, 1.25, 2.5, 5, 10, 20 and 40 mg/ml). The incubation was carried out in a waterbath at 37 °C for 3 h, the suspensions being gently mixed approximately every 30 min. Direct antiglobulin tests were carried out with the subjects' red cells and ortho 'broad-spectrum' antiglobulin reagent.

The sensitivity of red cells to cephalothin (i.e. the minimum concentration of the drug [mg/ml] sufficient to produce a positive direct Coombs test) varied from case to case (Figure 1). However, the statistical analysis of the data, performed with the MANN-WHITNEY U test[2], indicated that there was no difference between the groups of normal and azotemic subjects; on the contrary, the minimum sufficient concentration of the drug was significantly lower in the group of cirrhotic patients than in that of normal subjects ($p < 0.02$).

We then investigated the relationship between the sensitivity of red cells to cephalothin and some biochemical indices of the blood. For this purpose, a multiple regression 'step-wise' analysis was carried out, challenging the minimum sufficient concentration of cephalothin in turn with hematocrit, BUN, serum total protein level and concentration of protein fractions (albumin, α_1-, α_2-, β- and γ-globulins). The red cell sensitivity to the drug appeared to depend on the level of serum γ-globulins ($F = 10.11$, $p < 0.01$), the regression coefficient being negative (Figure 2).

We could not observe the relationship between the sensitivity of red cells to cephalothin and serum albumin concentration reported by LO BUGLIO[3].

The mechanism by which cephalothin produces a positive direct Coombs test is still uncertain: at present it seems likely that both some properties of the plasma and an alteration of the red cell play a role. The data presented here show that the level of serum γ-globulins influences the sensitivity of the blood to cephalothin. In so far as the erythrocyte alteration is concerned, previous experiments by MOLTHAN et al.[1], as well as results obtained in this laboratory, have shown that a positive direct Coombs test can be obtained not only by incubating cephalothin with whole blood, but also when washed erythrocytes are exposed to the drug, if suitable antiglobulin reagents are used. In the latter experimental condition, according to MOLTHAN et al.[1], the drug alters the α- and β-globulins of the cell membrane, so that these

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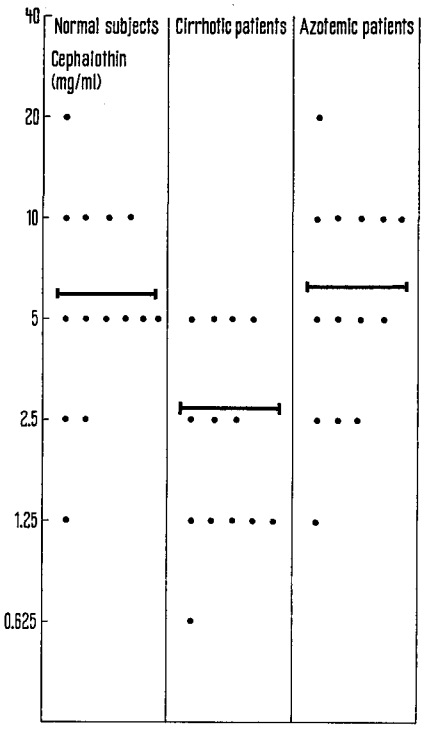

Fig. 1. Minimum concentration of cephalothin sufficient to produce a positive direct Coombs test in normal subjects and in cirrhotic and azotemic patients. Each dot represents a single case. The horizontal bar represents the mean of values.

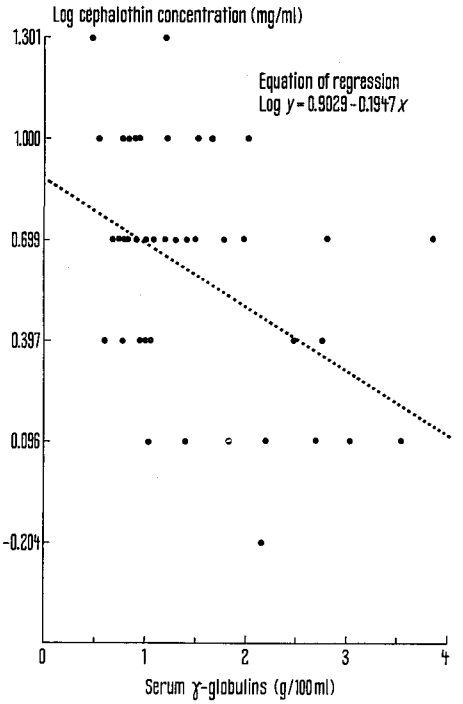

Fig. 2. Relationship between the log of the minimum concentration of cephalothin sufficient to produce a positive direct Coombs test (y) and the level of serum γ-globulins (x). Each dot represents a single case.

stromatic proteins become reactive with the anti-α and anti-β antibodies present in the antiglobulin reagent.

Further evidence that the drug alters the red cell membrane is given by the finding that cephalothin-treated red cells are susceptible to acid lysis[4] and display a low acetylcholinesterase activity[5].

From all this, it seems reasonable to conclude that the drug causes an alteration of the red cell surface with subsequent uptake of serum globulins[6].

Riassunto. Il test di Coombs diretto da cefalotina si produce a più bassa concentrazione dell'antibiotico se il sangue impiegato per l'esperimento in vitro è quello di pazienti affetti da cirrosi epatica classica piuttosto che quello di pazienti nefropatici o di soggetti normali. L'analisi statistica dei risultati ottenuti esaminando 41 differenti campioni di sangue dimostra che esiste una relazione inversa tra concentrazione minima di cefalotina sufficiente a determinare la positività del Coombs diretto e tasso di γ-globuline del siero. Pertanto, nel determinismo del Coombs diretto da cefalotina, oltre ad un'alterazione della membrana eritrocitaria, giocano un ruolo anche le caratteristiche proteiche del plasma.

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Indications of an Interspecific Transformation in *Allomyces*

The attempts of genetic transformations in moulds, *Neurospora crassa*[1,2] and *Penicillium chrysogenum*[3] have not been successful. We are unaware of examples of heterospecific transformation in fungi of the type reported in bacteria[4–6]. The resistance of fungi to the action of heterologous DNA may be due to the difficulty of transfer and passage across the thick cell wall, rich in chitin and of poor permeability. In this connection it is interesting to add that certain amino acids which are major components of the cell wall can impair the development of competence in *Bacillus subtilis*[7].

We think that *Allomyces* with its naked zoospores (plasmic membrane only) permits to overcome the difficulty of transfer. Additionally, at the time of encystment, the zoospores, in a fashion similar to the other Blastocladiales[8], loose their flagella by retraction, creating that way a solution of continuity or at least a weak point in the plasmic periphery. This could present a particularly