Cephalothin Positive Direct Coombs Test. Relationship to Serum Immunoglobulin Concentration

It has recently been reported that whole blood incubated in vitro with cephalothin under suitable experimental conditions gives a positive direct Coombs test[1,2]. It has been suggested that serological abnormality develops because of the adsorption of serum proteins to the red cell consequent upon an alteration of its membrane[3]. The minimum concentration of cephalothin sufficient to give the serological reaction (indicated as the susceptibility of the red cells to develop a direct Coombs test) has been observed to vary from subject to subject; a previous study carried out in this laboratory to determine the reason for this finding has shown that the minimum concentration of cephalothin sufficient to give a positive direct Coombs test is negatively correlated with serum γ-globulin concentration[2]. The aim of the present investigation is to establish whether the former is correlated with the serum content of γG, γA and γM globulins.

The investigation was carried out on the blood of 35 normal subjects, 34 cirrhotic patients and 16 patients suffering from myeloma.

Blood was drawn with acid-citrate-dextrose and incubated at 37 °C for 3 h with cephalothin solutions of different concentrations, as previously described[2]. At the end of the incubation period erythrocytes were washed 4 times with large volumes of saline. Direct Coombs test was carried out using commercial anti-whole-human serum and was scored from 1 + to 3 + depending upon the degree of microscopic agglutination. The level of γG, γA and γM globulins was determined by the single radial immunodiffusion method (immunoplates Hyland).

The statistical analysis of the data was carried out using the KRUSKAL-WALLIS test[4] or the correlation coefficient r according to the information required.

The minimum concentration of the drug sufficient to give a positive direct Coombs test was significantly lower than normal ($P < 0.01$) with the cirrhotic patients and with those suffering from myeloma, the 2 groups not differing significantly from each other (Figure 1).

The red cell susceptibility to cephalothin appeared to be negatively correlated with the concentrations of total γ-globulins ($r = -0.54$, $P < 0.001$) and of γG-globulins ($r = -0.69$, $P < 0.001$) (Figure 2), but not to those of γA and γM globulins.

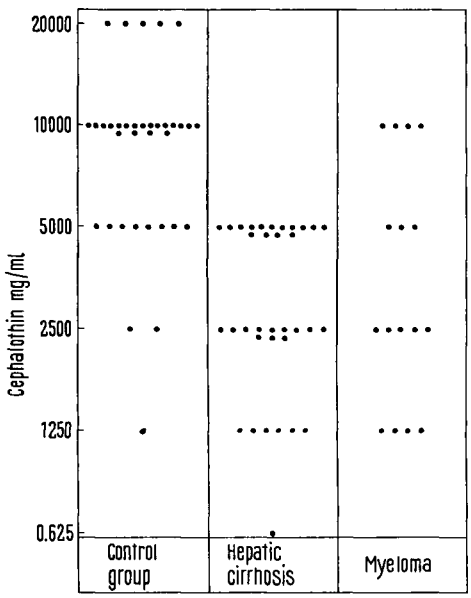

Fig. 1. Minimum concentration of cephalothin sufficient to produce a positive direct Coombs test in normal subjects, in cirrhotic patients and in patients suffering from myeloma. Each dot represents a single case.

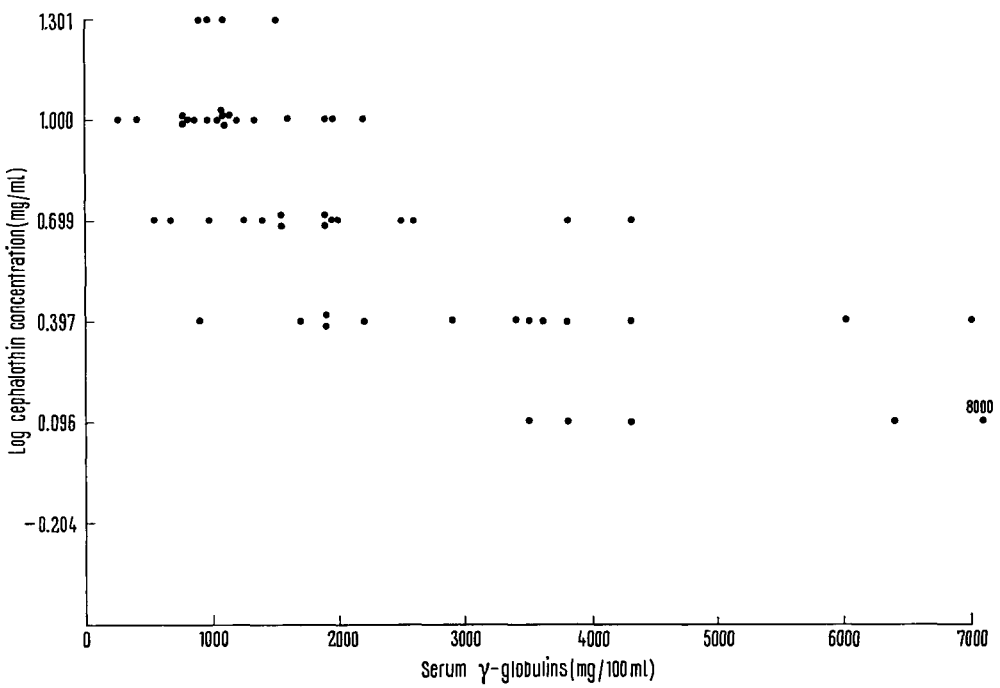

Fig. 2. Relationship between the log of the minimum concentration of cephalothin sufficient to produce a positive direct Coombs test and the level of serum γG-globulins. Each dot represents a single case.

The development of a positive direct Coombs test following in vitro incubation of whole blood with cephalothin is influenced by many variables, such as the concentration of the drug, the temperature and the period of incubation[1,2]. The foregoing experimental results indicate that in this respect the concentration of some serum proteins is also an important factor. The clinical interest of these findings resides in the possibility that the same source of variability operates also in vivo with patients receiving cephalothin; of these patients only a certain proportion, varying from 11 to 70% according to different authors[1,5-7], develop a positive direct Coombs test. In this respect it has been observed that when kidney function is impaired, the serologic abnormality tends to occur more frequently and this does not appear to depend on the dose of drug given and its serum concentration[6].

The results of the present study suggest the possibility of a useful investigation as to whether the development of a positive direct Coombs test in vivo may depend on the patients' serum protein concentration.

Riassunto. L'incubazione in vitro dell'antibiotico cefalotina con sangue intero determina la positività del test di Coombs diretto; la concentrazione di farmaco necessaria a determinare l'anormalità sierologica è correlata negativamente con il tasso di γ-globuline e di γG-globuline. Viene sottolineato l'interesse di tale reperto ai fini clinici, perchè potrebbe indicare la ragione per cui soltanto una parte dei soggetti ai quali viene somministrata la cefalotina sviluppa il test di Coombs diretto positivo.

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Red Cell Metabolism in Positive Direct Coombs Test After Cephalothin Therapy

It has recently been shown that most of the patients given cephalothin may develop a positive direct Coombs test (PDCT)[1-3]. As yet it has not been clearly established whether this serological finding is associated with an alteration of red cell metabolism. The aim of the present note is to report the results obtained from the study of some enzymatic and metabolic activities of red cells from patients with PDCT which appeared during cephalothin therapy.

Five patients out of 42 treated with cephalothin[4] (average 4 g daily i.v.) developed a PDCT and were included in the study. Direct antiglobulin test was carried out using commercial antihuman globulin serum (*ortho*) and was scored from 1+ to 3+ depending upon the degree of microscopic agglutination.

Routine haematologic investigations were performed with standard techniques. Reduced glutathione (GSH) was determined according to BEUTLER et al.[5], GSH stability to acetylphenylhydrazine according to BEUTLER[6], glucose-6-phosphatedehydrogenase (G-6-PD) according to KORNBERG and HORECKER[7], glutathione-reductase (GSSG-R) by the method of RACKER[8] slightly modified, piruvate kinase (PK) according to BÜCHER and PFLEIDERER[9], acetylcholinesterase (AChE) by the method of ELLMAN et al.[10], as previously described[11] the O_2 uptake in the presence of methylene blue according to OKA and PURANEN[12] and the glycolytic activity as lactic acid production in the presence of glucose as previously described[13].

Clinical and haematological data of patients studied are summarized in Table I; the results of the red cell enzymatic and metabolic activities investigated are reported in Table II. On the whole these results appear to be normal or high, the increase being probably an expression of the young red cell population present in the circulation. Only the stability of GSH to acetylphenylhydrazine in the patient S.S. is definitely subnormal; this result might be of technological origin since

Table I. Clinical and haematological data from 5 patients with PDCT after cephalothin therapy

Patient	Sex	Age	Disease	Haemoglobin g/100 ml	Reticulocytes ‰	Direct Coombs reaction	Cephalothin received at time of examination (g)
S.S.	♂	47	chronic pyelonephritis sepsis	5.2	57	++	60
A.S.	♂	27	chronic glomerulonephritis sepsis	4.7	14	+	60
M.F.	♂	42	polycystic kidney sepsis	7.6	23	+	47
I.C.	♂	25	agranulocytosis bronchopneumonia	10.5	38	++	30
P.M.	♀	83	bronchopneumonia	9.7	33	+	20

The titre of PDCT ranged from 1:1 to 1:4.