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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1 L. MOLTHAN, M. M. REIDENBERG and M. F. EICHMAN, New Engl. J. Med. *277*, 123 (1967).
2 S. FERRONE, F. MERCURIALI and G. SIRCHIA, Experientia *25*, 78 (1969).
3 S. FERRONE, A. ZANELLA, F. MERCURIALI and G. SIRCHIA, Experientia *26*, 1255 (1970).
4 W. A. KRUSKAL and W. A. WALLIS, J. Am. statist. Ass. *47*, 583 (1952).
5 H. R. GRALNICK, L. D. WRIGHT and M. H. MCGINNISS, J. Am. med. Ass. *199*, 725 (1967).
6 R. L. PERKINS, C. E. MENGEL and S. SASLAW, Proc. Soc. exp. Biol. Med. *129*, 397 (1968).
7 S. FERRONE, A. ZANELLA and M. SCALAMOGNA, Experientia, in print (1971).

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.

Table II. Data from red cell studies on 5 patients with PDCT after cephalothin therapy

Patient	GSH mg/100 ml of blood		G-6-PD	GSSG-R	AChE	PK	O_2 uptake	Lactic acid production
	Before incubation with acetyl-phenyl-hydrazine	After incubation with acetyl-phenyl-hydrazine	mU/10^9 erythrocytes	mU/10^9 erythrocytes	mU/10^9 erythrocytes	mU/10^9 erythrocytes	QO_2 $\mu l\ O_2$/mg/h	γ/mg/h
S.S.	56.0	16.6	61.8	59.7	728.4	733.1	0.916	–
A.S.	56.6	58.3	351.4	115.2	538.3	–	0.620	–
M.F.	64.1	66.3	257.7	123.3	628.8	–	0.695	0.964
I.C.	87.5	67.5	188.3	94.7	459.5	726.2	0.403	5.268
P.M.	71.9	58.6	156.8	85.5	590.3	268.4	1.066	0.338
Normal values (Mean $\pm$ 2 S.D.)	72.5 $\pm$ 21.6	69.1 $\pm$ 22.6	148.0 $\pm$ 64.6	71.7 $\pm$ 39.4	615.3 $\pm$ 212.4	298.2 $\pm$ 114.6	0.637 $\pm$ 0.215	0.918 $\pm$ 0.786

it has been shown by SABINE[14] and by LAWRENCE and GROSSMAN[15] that the red cell GSH stability of normal blood becomes abnormal when the haematocrit of the blood sample is low.

No relationship could be found between the values of the erythrocyte enzymatic and metabolic activities investigated and the intensity of the antiglobulin test.

The foregoing results have not produced evidence of metabolic abnormality of erythrocytes from patients with PDCT after cephalothin therapy. Similarly PERKINS et al.[3] did not observe any significant change of osmotic fragility, auto-haemolysis, ATP and glycolytic enzymes levels in erythrocytes from Rhesus monkeys in which PDCT was induced with cephalothin. Moreover it has previously been reported from this laboratory that the survival of normal human red cells with a PDCT after in vitro treatment with cephalothin was within normal limits[16].

All these data suggest that in man the PDCT associated with cephalothin treatment does not apparently determine any deleterious effect on red blood cells. This conclusion contrasts with the results previously obtained by incubating normal human red cells with cephalothin in vitro; under these experimental conditions altered red cells display low AChE activity and reduced O_2 uptake in the presence of methylene blue[17,18].

However, the concentrations of the drug necessary to determine alteration of the erythrocyte metabolism in vitro are much higher than those likely to be found in clinical use and this could explain the above-mentioned discrepancy between the in vivo and in vitro findings.

The results of the present investigation invite comparison with similar studies carried out in other conditions where PDCT is determined by antibodies. These have shown that AChE activity is subnormal in auto-immune haemolytic anaemia[19–21] and in ABO haemolytic disease of the newborn[22,23].

The different behaviour of red cell metabolism in these immuno-haemolytic disorders from that in cephalothin-induced PDCT seems to be a further demonstration of the different mechanisms responsible for the development of the serologic abnormality in these two conditions.

Riassunto. In cinque soggetti che avevano sviluppato il test di Coombs diretto positivo durante la terapia con cefalotina sono state studiate alcune attività enzimatiche e metaboliche eritrocitarie. Nel complesso queste sono risultate normali o aumentate, l'incremento essendo probabilmente espressione della concomitante reticolocitosi. I risultati sono discussi ed è concluso che la terapia con cefalotina nell'uomo non sembra determinare alcun effetto dannoso sul metabolismo eritrocitario.

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[1] L. MOLTHAN, M. M. REIDENBERG and M. F. EICHMAN, New Engl. J. Med. *277*, 123 (1967).
[2] H. R. GRALNICK, L. D. WRIGHT JR. and M. H. MCGINNIS, J. Am. med. Ass. *199*, 725 (1967).
[3] R. L. PERKINS, C. E. MENGEL and S. SASLAW, Proc. Soc. exp. Biol. Med. *129*, 397 (1968).
[4] Keflin, kindly supplied by Eli Lilly and Co., Indianapolis, USA.
[5] E. BEUTLER, O. DURON and B. M. KELLY, J. Lab. clin. Med. *61*, 882 (1963).
[6] E. BEUTLER, J. Lab. clin. Med. *49*, 84 (1954).
[7] A. KORNBERG and B. L. HORECKER, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), vol. 1, p. 323.
[8] E. RACKER, J. biol. Chem. *217*, 855 (1955).
[9] TH. BÜCHER and G. PFLEIDERER, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), vol. 1, p. 435.
[10] G. L. ELLMAN, K. D. COURTNEY, V. ANDRES JR. and R. M. FEATHERSTONE, Biochem. Pharm. *7*, 88 (1961).
[11] G. SIRCHIA, S. FERRONE and F. MERCURIALI, Haemat. lat. *11*, 363 (1968).
[12] M. OKA and J. PURANEN, Acta haemat. *28*, 119 (1962).
[13] G. SIRCHIA, S. FERRONE, R. MILANI and F. MERCURIALI, Blood *28*, 98 (1966).
[14] J. C. SABINE, Br. J. Haemat. *10*, 477 (1964).
[15] C. LAWRENCE and R. GROSSMAN, J. Lab. clin. Med. *65*, 102 (1965).
[16] S. FERRONE and G. SIRCHIA, J. Am. med. Ass. *206*, 378 (1968).
[17] S. FERRONE, A. ZANELLA, F. MERCURIALI and C. PIZZI, Europ. J. Pharmac. *4*, 211 (1968).
[18] F. HERZ, E. KAPLAN and D. A. SEVDALIAN, Acta haemat. *41*, 94 (1969).
[19] K. R. TANAKA, W. W. VALENTINE and A. S. SCHNEIDER, Clin. Res. *12*, 110 (1964).
[20] V. BRABEC, J. BICANOVÁ, B. FRIEDMANN, M. KOUT, L. MIRČEVOVÁ, J. PALEK, M. VOPATOVÁ und V. VOLEK, Acta haemat. *34*, 88 (1965).
[21] G. SIRCHIA, S. FERRONE, F. MERCURIALI and A. ZANELLA, Br. J. Haemat. *19*, 411 (1970).
[22] E. KAPLAN, F. HERZ and K. S. HSU, Pediatrics *33*, 205 (1964).
[23] G. SIRCHIA, A. M. SIRONI, S. FERRONE and G. MASERA, Boll. Soc. ital. Emat. *13*, 197 (1965).