

## Red Cell Acetylcholinesterase in ABO Haemolytic Disease of the Newborn

It has recently been shown<sup>1</sup> that in most cases of ABO haemolytic disease of the newborn the red cell acetylcholinesterase (AChE) activity is below normal. Other intracellularly located enzymes, such as lactic dehydrogenase, glucose-6-phosphate dehydrogenase, acid phosphatase and inorganic pyrophosphatase, as well as the stromal enzymes alkaline phosphatase and adenosine triphosphatase, have been found to be either normal or increased<sup>1</sup>.

The pathogenesis of the enzyme defect is obscure: the role of anti-A and anti-B isoantibodies in producing this abnormality is suggestive, but not proved. Attempts to reproduce a change in AChE activity by coating normal erythrocytes with ABO isoantibodies in vitro and in vivo have been unsuccessful<sup>1</sup>.

In order to elucidate the importance of 'immune' anti-A and anti-B in determining the enzyme defect, we have investigated if the elution of the antibody might influence the red cell AChE activity. The red cell AChE activity was measured before and after partial elution of the antibody in 14 newborns with Coombs positive ABO haemolytic disease and, for comparison, in 18 newborns with Rh disease and in 24 normal infants.

The elution was carried out according to LANDSTEINER and MILLER<sup>2</sup> by heating the red cells at 56°C for 30 min; normal red cells were subjected to the same procedure. The AChE activity was determined by manometric Warburg technique according to DE SANDRE et al.<sup>3</sup>; it was expressed in terms of  $\mu\text{l}$  of  $\text{CO}_2$  liberated/mg dry weight of red cells/h ( $\text{QCO}_2$ ). Results are presented in the Table.

The AChE activity of untreated red cells in ABO disease is significantly lower than in Rh disease and in normal newborns ( $P < 0.05$ ). After heating, in every case of ABO disease a significant increase of the enzyme activity was observed ( $P < 0.05$ )<sup>4</sup>; on the contrary, the AChE mean value diminished in the other conditions ( $P < 0.05$ )<sup>4</sup>, presumably as a consequence of the heat lability of the enzyme<sup>5</sup>.

The above data suggest that in ABO disease the isoantibody action on the red cell membrane plays a part in determining the AChE deficiency; the increase of the red cell enzyme activity after heating might be the expression of a reactivation of AChE consequent to the partial removal of the antibody.

**Riassunto.** E' stata studiata l'acetilcolinesterasi eritrocitaria (AChE) in 24 neonati normali, in 18 casi di malattia emolitica del neonato da isoimmunizzazione Rh (MEN Rh) ed in 14 casi di MEN ABO con Coombs diretto positivo. L'AChE in quest'ultimo gruppo è risultata significativamente più bassa di quella ottenuta negli altri due gruppi. Il riscaldamento delle emazie a 56°C per 30 min, effettuato al fine di ottenere una parziale eluzione dello anticorpo, ha determinato un significativo aumento della attività enzimatica in ogni caso di MEN ABO ed una significativa diminuzione nelle altre due condizioni. Tale rapporto suggerisce che l'anticorpo immune responsabile della MEN ABO può giocare un ruolo nel determinare il difetto enzimatico eritrocitario riscontrato in tale affezione.

S. FERRONE, A. ZANELLA and G. SIRCHIA

*Istituto di Patologia Speciale Medica (II),  
University of Milano (Italy), 13 December 1967.*

Effect of heating on the red cells AChE activity in normal infants and in ABO and Rh haemolytic disease of the newborn

	No. of cases	Red cell AChE ( $\text{QCO}_2$ )	
		Before heating	After heating
Normal	24	20.53 $\pm$ 0.80 <sup>a</sup>	16.07 $\pm$ 1.41
ABO haemolytic disease	14	10.97 $\pm$ 0.69	13.74 $\pm$ 1.33
Rh haemolytic disease	18	22.70 $\pm$ 0.90	20.00 $\pm$ 1.36

<sup>a</sup> Mean  $\pm$  standard error.

<sup>1</sup> E. KAPLAN, F. HERZ and K. S. HSU, *Pediatrics* 33, 205 (1964).

<sup>2</sup> K. LANDSTEINER and C. P. MILLER, *J. exp. Med.* 42, 853 (1925).

<sup>3</sup> G. DE SANDRE, G. GHIOTTO and G. MASTELLA, *Acta med. patav.* 16, 291 (1956).

<sup>4</sup> In order to evaluate the effect of heating on red cell AChE, statistical analysis was performed on the differences between the values obtained before and after heating. This approach was followed in order to eliminate the variability due to the different subjects.

<sup>5</sup> K. B. AUGUSTINSON, in *The Enzymes. Chemistry and Mechanism of Action* (Eds. J. B. SUMMER and K. NYRBAËCK, Academic Press Inc., New York 1950), Vol. 1, p. 443.

## Cephalothin-Treated Normal Red Cells: A New Type of PNH-like Cells

MOLTHAN et al.<sup>1</sup> and GRALNICK et al.<sup>2</sup> have recently reported a positive direct Coombs test in a large number of patients receiving the antibiotic cephalothin. Moreover, in vitro studies<sup>1</sup> have shown that under suitable experimental conditions, the addition of cephalothin to washed normal red cells produces a positive Coombs test of non-y type. According to MOLTHAN et al.<sup>1</sup> this is a consequence of alteration of some cell-membrane proteins by the drug. The same authors also noted that the exposure of washed red cells to higher doses of cephalothin (final concentration 50 mg/ml) caused severe lysis.

Since it has been previously demonstrated that certain proteolytic enzymes<sup>3</sup> and sulphhydryl compounds<sup>4</sup> which alter the red cell membrane can, under suitable condi-

tions, also render the cells susceptible to lysis in acidified serum (PNH-like cells), we have performed some in vitro lysis tests on cephalothin-treated normal red cells.

The blood used for the experiment was withdrawn into ACD from healthy donors. Four volumes of a 50% sus-

<sup>1</sup> H. MOLTHAN, M. M. REIDENBERG and M. F. EICHMAN, *New Engl. J. Med.* 277, 123 (1967).

<sup>2</sup> H. R. GRALNICK, H. D. WRIGHT JR. and M. H. MCGINNIS, *J. Am. med. Ass.* 199, 725 (1967).

<sup>3</sup> S. YACHNIN, M. T. LAFORET and F. H. GARDNER, *Blood* 17, 83 (1961).

<sup>4</sup> G. SIRCHIA, S. FERRONE and F. MERCURIALI, *Blood* 25, 502 (1965).