Red Cell Acetylcholinesterase in ABO Haemolytic Disease of the Newborn

It has recently been shown[1] that in most cases of ABO haemolytic disease of the newborn the red cell acetylcholinesterase (AChE) activity is below normal. Other intracellulary 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[1].

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[1].

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[2] by heating the red cells at 56°C for 30 min; normal red cells were subjected to the same procedure. The AChE activity was determinated by manometric Warburg technique according to DE SANDRE et al.[3]; it was expressed in terms of μl of CO_2 liberated/mg dry weight of red cells/h (QCO_2). Results are presented in the Table.

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 (QCO_2) Before heating	After heating
Normal	24	20.53 ± 0.80[a]	16.07 ± 1.41
ABO haemolytic disease	14	10.97 ± 0.69	13.74 ± 1.33
Rh haemolytic disease	18	22.70 ± 0.90	20.00 ± 1.36

[a] Mean ± standard error.

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 ($P < 0.05$)[4]; on the contrary, the AChE mean value diminished in the other conditions ($P < 0.05$)[4], presumably as a consequence of the heat lability of the enzyme[5].

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 reperto suggerisce che l'anticorpo immune responsabile della MEN ABO può giocare un ruolo nel determinare il difetto enzimatico eritrocitario riscontrato in tale affezione.

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

MOLTHAN et al.[1] and GRALNICK et al.[2] have recently reported a positive direct Coombs test in a large number of patients receiving the antibiotic cephalothin. Moreover, in vitro studies[1] have shown that under suitable experimental conditions, the addition of cephalothin to washed normal red cells produces a positive Coombs test of non-γ type. According to MOLTHAN et al.[1] 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[3] and sulphydryl compounds[4] which alter the red cell membrane can, under suitable conditions, 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-

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pension in saline of red cells thoroughly washed in saline were added to 1 volume of a 200 mg/ml solution in saline of cephalothin (Keflin Lilly) (final concentration 40 mg/ml), the mixture was incubated in a waterbath at 37 °C for 3 h and gently mixed approximately every 30 min. The concentration of cephalothin solution, the drug/red cells ratio and the incubation time and temperature were adopted after they had proved to be optimal in a series of preliminary experiments. At the end of the incubation period, the red cells were repeatedly washed with a large volume of saline until the supernatant was completely free from hemoglobin. The in vitro lysis tests were carried on as previously described[4], and the Coombs tests were performed with different anti-γ and anti-non-γ reagents.

In addition to a positive non-γ (Hyland) Coombs test, as observed by MOLTHAN et al.[1], cephalothin-treated red cells give a positive Ham test: i.e. they lyse in slightly acidified fresh compatible normal serum (pH 6.5), while lysis does not appear if the above medium is previously heated at 56 °C for 30 min to destroy complement. Depending upon the different normal sera used, a varying susceptibility to lysis of the same altered red cells was observed: likewise, altered red cells from different healthy donors underwent different degrees of lysis when incubated in the same normal serum.

Cephalothin-treated red cells also give a positive cold-antibody hemolysis test. They are thus PNH-like, in that they behave in certain in vitro lysis tests in the same way as do the red cells of paroxysmal nocturnal hemoglobinuria (PNH). Cephalothin, a sodium salt of 7-(thiophene-2-acetamido) cephalosporanic acid, resembles certain chemically-different substances (proteolytic enzymes, sulphydryl compounds) in its capacity to cause a PNH-like lesion in the normal red cell membrane. The mechanism by which different substances can render normal red cells similar in some respects to PNH-cells is unknown. However, cephalothin seems to alter the cell membrane in a new way, for cephalothin-treated red cells react with antiglobulin serum while cells treated with proteolytic enzymes or sulphydryl compounds do not. This observation strongly suggests that this type of PNH-like cell is produced by an alteration to some of the proteins of the cell membrane and supports the hypothesis that the defect which occurs spontaneously in PNH is located in the protein moiety of the red cell stroma[5,6].

Riassunto. Emazie umane normali trattate con cefalotina, in opportune condizioni sperimentali, si comportano in alcuni test di emolisi in vitro (test di emolisi acida, test di sensibilità agli isoanticorpi freddi) in modo simile alle emazie della emoglobinuria parossistica notturna (EPN) (emazie simil-EPN). A differenza di altri tipi di emazie simil-EPN, queste emazie danno anche un test di Coombs diretto positivo, di tipo non γ. Viene avanzata l'ipotesi che la lesione responsabile del comportamento simil-EPN di queste emazie sia da ricercare in una alterazione delle proteine stromali.

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Urinary Output of Rats in Response to Subcutaneous Injections of Adrenaline

The effect of various doses of adrenaline on the urinary output of water and electrolytes in rats has been confusing. Early workers[1–6] found that injections of adrenaline in the range of 32–100 μg/100 g body weight caused diuresis and natriuresis in rats and concluded that these were the only effects of injected adrenaline. However, LOCKETT and her co-workers[7–9] further investigated these effects in rats, paying special attention to threshold effects. They used doses much smaller than those used previously. Threshold doses of 2.5 μg adrenaline/100 g body weight given s.c. up to 25 μg/100 g body weight, were found to be antidiuretic and sodium retaining. This antidiuresis was not affected by adrenalectomy, by partial hepatectomy, by removal of the posterior pituitary gland or by section of the afferent nerves from the injection site. They further showed that neither the systemic mean arterial pressure nor the renal clearance of inulin were affected by these doses. It was also demonstrated that doses ranging from 50–200 μg/100 g body weight caused a diuresis. However, they did not indicate whether there was an increase in sodium excretion. In this communication, experimental evidence is presented in an attempt to correlate the renal effects of adrenaline that have been reported.

Four separate groups of Wistar rats were used. They were injected s.c. with adrenaline in doses ranging from 2.5–40.0 μg/100 g body weight after an oral water load and observed over the following hour. The experimental technique has been previously described[10]. Each rat acted as its own control in the cross-over tests. The Table shows the results of the experiments, each carried out separately. Experiments 1 and 2 showed quite clearly that with a dose of 2.5 μg/100 g body weight, there was an antidiuresis as well as sodium retention. These effects were maximal with 5.0 μg/100 g body weight ($P < 0.01$

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