Effect of Acetylcholine on the Osmotic Fragility of Papain-Treated and Untreated Human Red Blood Cells

Acetylcholine (ACh) was found to affect the permeability of red blood cells to ions[1] and to change their osmotic fragility[2]. These cells are provided with acetylcholinesterase (AChE) which is located on the external surface of their membranes[3]. The AChE is inactivated by treating the cells with proteolytic enzymes[3,4]. Such a treatment was reported to have no effect on the choline transport and on the sodium pump of the cells examined in the absence of ACh[4].

The purpose of the present study was to examine the effect of inactivation of the AChE of human red blood cells by papain on the sensitivity of these cells to the hemolytic effect of very high concentrations of ACh.

Papain-treated and untreated erythrocytes obtained from clinically normal adults were incubated at room temperature with acetyl choline chloride (Sigma) in a total volume of 1 ml containing: 0.25 ml of washed packed erythrocytes, 0.35 ml of 0.85% NaCl solution and 0.4 ml

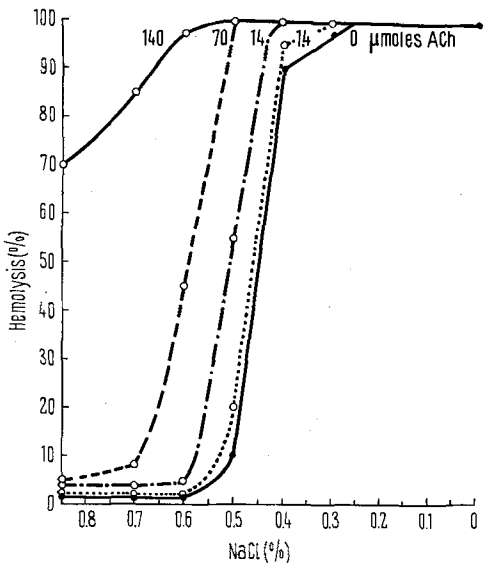

Fig. 1. The osmotic fragility of erythrocytes after incubation for 20 h without ACh (●—●) or with 1.4 (○...○), 14 (○-.-○), 70 (○---○) and 140 (○—○) µmoles ACh/ml. A complete hemolysis was frequently obtained at the incubation stage in the medium containing 0.14 *M* ACh.

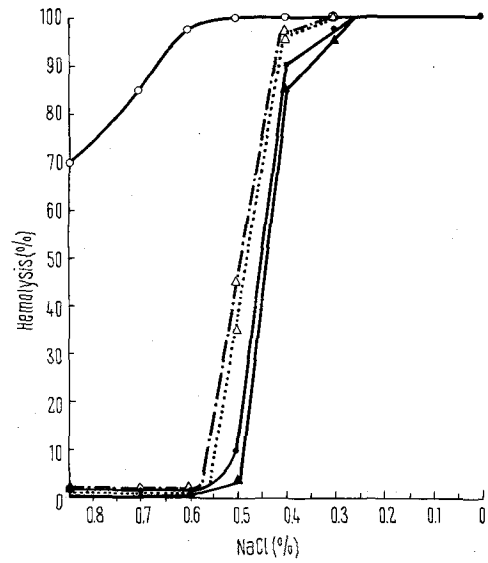

Fig. 2. The osmotic fragility of erythrocytes after incubation with 0.14 *M* ACh for 30 min (△...△), 60 min (△-.-△) and 20 h (○—○) and without it for 30–60 min (▲—▲) and 20 h (●—●).

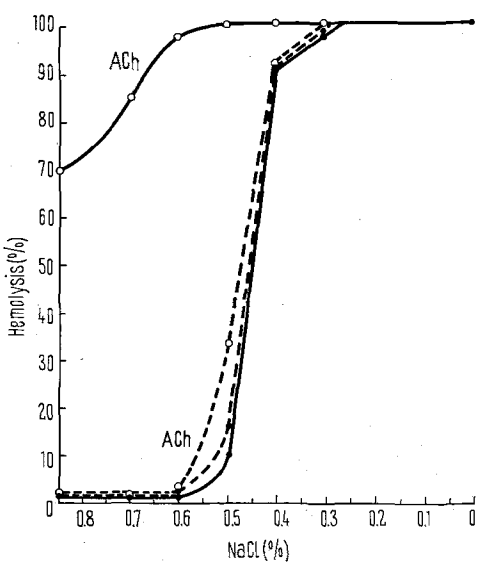

Fig. 3. The osmotic fragility, after 20 h incubation, of papain-treated erythrocytes with 0.14 *M* ACh (○----○) and without it (●---●) and of untreated cells with 0.14 *M* ACh (○—○) and without it (●—●).

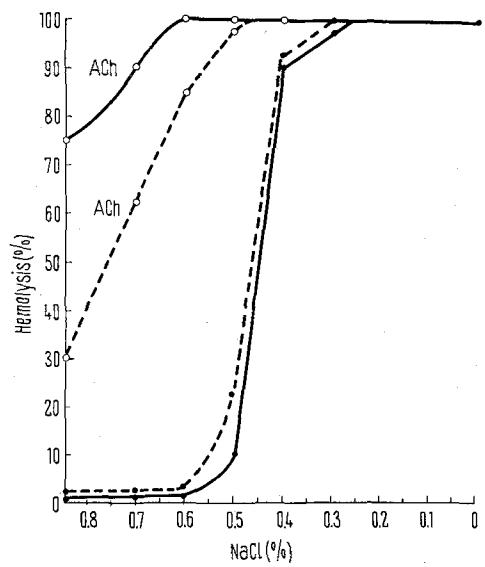

Fig. 4. The osmotic fragility, after 20 h incubation in the presence of plasma cholinesterase, of papain treated erythrocytes with 0.14 *M* ACh (○--○) and without it (●--●) and of untreated cells with 0.14 *M* ACh (○—○) and without it (●—●). (○—○) represents also the papain-treated cells incubated for 20 h with ACh in the presence of purified bovine AChE or of AChE of the electric eel.

of Na-K-phosphate buffer (0.15 M for lower and 0.25M for higher ACh concentrations) at pH 7.5. After exposure to ACh (time and concentrations as indicated in the Figures), the cells were washed 3 times and resuspended in 0.75 ml of 0.85% NaCl solution. 0.1 ml aliquots of the cell suspension were introduced into distilled water and NaCl solutions (3 ml) at concentrations of: 0.85, 0.7, 0.6, 0.5, 0.4 and 0.3%. After 1 h at room temperature, the cell suspensions were centrifuged and the hemoglobin content in the supernatant fluids was determined using a Klett photocolorimeter (filter No. 54).

The osmotic fragility of the erythrocytes incubated for 20 h with ACh or acetylthiocholine (AThCh) at concentrations of $10^{-3}M$ to $10^{-2}M$ was definitely increased. Higher concentrations of these 2 compounds brought about a greater increase of the cell fragility up to a pronounced or a complete hemolysis, induced at the incubation stage (Figure 1). Incubation of the erythrocytes for 20 h with very high concentration ($1.4 \times 10^{-1}M$) of succinylcholine (SCh) and butyrylcholine (BCh) caused only a small increase in the osmotic fragility of the cells. The time-dependent hemolytic effect (Figure 2) was due to the production of acetic acid accompanied by a decrease in the pH of the medium, which was not prevented in isotonic conditions. Papain treatment of the red blood cells was found to abolish this hemolytic effect (Figure 3).

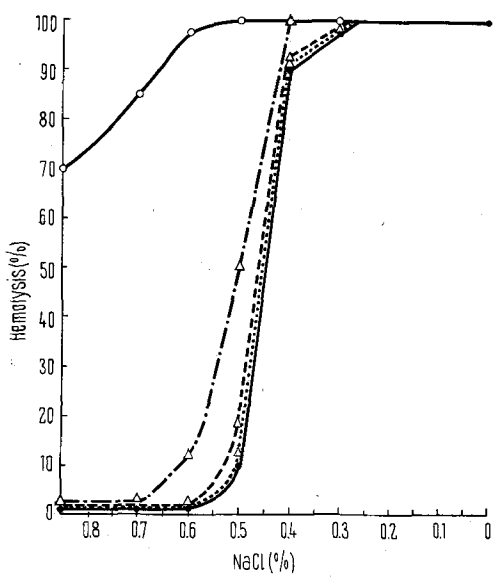

Fig. 5. The osmotic fragility of red blood cells after incubation for 20 h with 0.14 M concentrations of: sodium acetate (△....△), choline chloride (△--△), both acetate and choline (△-·-△), ACh (○—○) and without them (●—●).

External AChE from bovine erythrocytes (Sigma, type I) or from electric eel (Sigma, type V), or plasma cholinesterase, added to the incubation medium of the papain-treated cells, restored the hemolysis by ACh (Figure 4) and by AThCh. In an incubation medium contaning plasma cholinesterase, a complete hemolysis was induced by $1.4 \times 10^{-1}M$ butyrylcholine, too. As this cholinesterase catalyses a rapid hydrolysis of butyrylcholine, the butyric acid produced was involved in the hemolytic effect in this case.

Na-acetate or choline chloride at $1.4 \times 10^{-1}M$ concentration had no significant effect, but in the presence of both of them the osmotic fragility of the cells was definitely augmented (Figure 5). Addition of acetic acid at the same molar concentration, to the buffer containing incubation medium, lowered the pH of the medium to pH 4–5 and induced a complete hemolysis.

The foregoing experimental results indicate that inactivation of the erythrocyte AChE by papain protects the cells from the profound hemolytic effect of very high concentrations of ACh and AThCh.

Résumé. L'hémolyse des globules rouges, incubés avec de fortes concentrations d'acétylcholine en conditions isotoniques est due à l'acide acétique libéré dans le milieu. Cette hémolyse est complètement inhibée par l'inactivation de l'acétylcholinestérase érythrocytaire avec la papaïne.

NECHAMA GILBOA-GARBER and LEA MIZRAHI[5]

Section of Biochemistry, Department of Life Sciences, Bar-Ilan University, Ramat-Gan (Israel), 7 June 1971.

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Aberrant Thymus Tissue in Rat and Mouse Thyroid

One of the most important discoveries of modern immunology is the role of the thymus in the control of the immune response. Many studies have shown the function of this gland by removing it, especially from new-born animals, where its role is much more prominent than in adults. We want now to report aberrant thymus tissue located near and in the thyroid gland in mice and rats. In these animals the thymus is in the thoracic cavity, ventral to the base of the heart and aortic arch.

The thymus is a lymphoid organ, differing from lymph nodes in being epithelial in origin and character and in having no sinusoids. The thymic lobes are epithelial thickenings in the region of the 3rd and 4th pharyngeal pouches in 11-day-old mouse embryos. During the 15th day these epithelial vesicles separate from the pharyngeal epithelium and come to lie anterolateral to the heart, and during the subsequent 4 days they grow, migrate posteromedially and become lymphoidal[1]. The rodent thymus has a dense cortex, surrounding a pale, irregularly arranged medulla. The cortex is composed of densely packed masses of small lymphocytes called 'thymocytes'.

More than 40 years ago, DE WINIWARTER[2] described the possibility that thyroid and parathyroid glands include thymic tissue, which might be confused with tangential sections of the thyroid follicles. He thought that the thyroid and parathyroid cells could be locally

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