Vitamin E, catalase, manganous or cobaltous ions and dithiothreitol protect against Tween 20-induced hemolysis of vitamin E-deficient chick and kid erythrocytes

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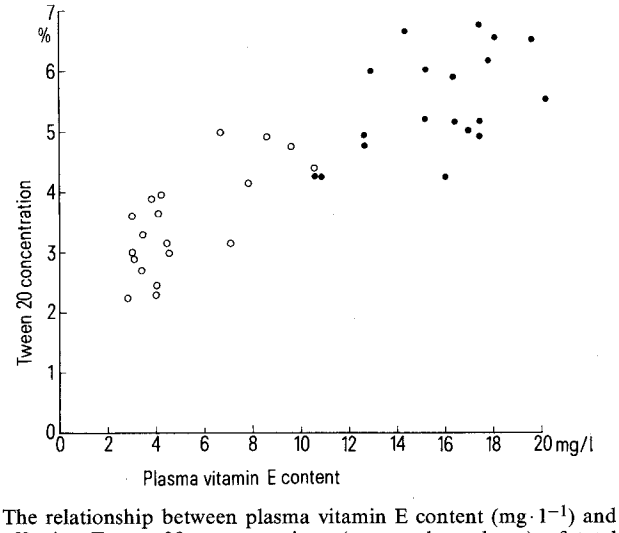
Summary. The effective Tween 20 concentration at which 70% hemolysis was achieved in vitro correlated with the plasma vitamin E content of chicks (r=0.85). Addition of catalase, $MnCl_2$, $CoCl_2$ or dithiothreitol in vitro showed significant protection against the hemolysis induced by Tween 20 in vitamin E-deficient chick and kid erythrocytes.

We have reported[1,2] that extensive in vitro hemolysis, induced by 1–2.5% Tween 20 in vitamin E-deficient kids, goats and chicks, or induced by 0.001% Tween 20 with 1.2 mM ascorbate and 5.3 mM azide in vitamin E-deficient rats, can be prevented either by the inclusion of vitamin E in the cells or by in vitro addition of 0.25–0.8 mM dithiothreitol (DTT). Tween 20 (polyoxyethylene sorbitan monolaurate) is a nonionic surfactant and can cause hemolysis like hydrogen peroxide or dialuric acid. Deficiency of vitamin E is not essential for the hemolysis[2,3], but vitamin E-deficient cells are much more readily hemolyzed than vitamin E-sufficient cells. In this work we examined the effect of different concentrations of Tween 20 in causing the hemolysis of chick erythrocytes of different vitamin E status. We also examined the protective effects of several substances against the Tween 20-induced hemolysis of vitamin E-deficient chick and kid erythrocytes.

Materials and methods. 38 male chicks (White Rock × White Leghorn) of 2 days of age were fed either a vitamin E-deficient or a vitamin E-supplemented diet for 6 weeks. These diets consisted of casein 20%, sucrose 63.9%, lard 5% lecithin 1%, cellulose 3% and vitamin-mineral mixture 7.1%. Mineral and vitamin contents were the same as those given by NRC feeding standards for chicks[4] and rats[5], respectively. The vitamin E-supplemented diet also contained 30 mg dl-α-tocopheryl acetate per kg. Japanese meat-type kids of 1 week of age were also fed either vitamin E-deficient or vitamin E-supplemented milk for 7 weeks, as described previously[1]; the vitamin E-supplemented milk contained 200 mg dl-α-tocopheryl acetate per kg dry matter.

Heparinized blood samples were taken by cardiac puncture and from the jugular vein in chicks and kids, respectively. 25 vol. of saline-phosphate buffer (pH 7.4) was added to 1 vol. of chick blood, and the mixture was centrifuged and the supernatant discarded. This washing procedure was repeated and the cell pellet was resuspended with the same volume of saline-phosphate buffer. 4–20% Tween 20 solutions (Wako Pure Chemical Co.) were made with 0.9% NaCl and stored at 4 °C. 0.4 ml of the cell suspension, 0.1 ml of 0.9% NaCl and 0.5 ml of Tween 20 solution were mixed and incubated at 37 °C for 15 min. Immediately after the incubation, the mixture was cooled by ice and 2 ml of cold buffer was added. Percentage hemolysis was measured as shown previously[1,2]. For each blood sample the effective Tween 20 concentration at which 70% hemolysis was achieved was intrapolated from a curve by plotting the percentage hemolysis versus Tween 20 concentration of incubation medium. In the experiment to examine the effects of adding various substances, 0.4 ml of chick or kid erythrocyte suspension, 0.1 ml of 0.9% saline containing the added substance and 0.5 ml of 7.5% Tween 20 solution were incubated at 37 °C for 15 min. Catalase (bovine liver), superoxide dismutase (bovine blood) and albumin (bovine serum) were from the Sigma Chemical Co. (USA).

Plasma vitamin E was analyzed as follows. 50–100 µl of plasma was diluted to 1 ml with water, and 3 ml of ethanol and 5 ml of hexane were added. After shaking for 2 min, the fluorescence of the hexane layer was read at 340 nm with activation at 295 nm using Aminco-Bowman spectrophotofluorometer. The statistical test method[6] was used for establishing the least significant difference.



The relationship between plasma vitamin E content (mg · l^{-1}) and effective Tween 20 concentrations (percent by volume) of total incubation medium at which 70% hemolysis is achieved. Open circles show the chicks fed vitamin E-deficient diet and black circles show the chicks fed vitamin E-supplemented diet.

Protective effects of several substances against the Tween 20-induced hemolysis of vitamin E-deficient chick and kid erythrocytes on incubation with 3.75% Tween 20 at 37 °C for 15 min

	Percentage hemolysis (%)	
	Chick erythrocytes	Kid erythrocytes
Animals fed vitamin E-deficient diets*		
No addition	91 (97–80)	76 (85–71)
+ Catalase (100 µg/ml)**	44 (62–26)	4 (5–3)
+ Catalase (50 µg/ml)	48 (60–26)	3 (4–2)
+ Albumin (100 µg/ml)	97 (99–93)	71 (77–67)
+ Glucose (2 mg/ml)	95 (97–93)	71 (79–65)
+ $MnCl_2$ (0.25 mM)	17 (18–17)	4 (8–2)
+ $CoCl_2$ (0.25 mM)	33 (38–24)	9 (15–4)
+ Dithiothreitol (0.6 mM)	14 (18–10)	2 (2–1)
Animals fed vitamin E-supplemented diets*		
No addition	15 (16–15)	2 (3–2)

Data are shown as mean (maximum value – minimum value) for 3 animals used. The least significant differences at the 5% level were 16.8 and 7.8 in chick and kid erythrocytes, respectively. *Plasma vitamin E contents of chicks fed vitamin E-deficient and vitamin E-supplemented diet were 4 ± 0.3 and 18 ± 2 mg · l^{-1}, respectively, and those of kids fed vitamin E-deficient and vitamin E-supplemented diet were 0.7 ± 0.2 and 6.3 ± 1.0 mg · l^{-1}, respectively. **The figures in parentheses indicate final concentrations of added substances in total incubation medium.

Results and discussion. The figure shows the relationship between plasma vitamin E content (X, mg/l) and the effective Tween 20 concentration (Y, %) to induce 70% hemolysis. The correlation coefficient was 0.85, and an equation $Y=0.18\ X+2.57$ was deduced. These data showed the antagonistic relationship between vitamin E and Tween 20. The hemolytic power of Tween 20 was concentration-dependent, and vitamin E-deficient erythrocytes increased in their susceptibility to hemolysis at lower Tween 20 concentrations. By proper selection of the Tween 20 concentration a clear distinction between vitamin E-deficient cells and vitamin E-sufficient cells could be made.
In the table, protective effects of various substances against Tween 20 hemolysis are shown. The addition of catalase significantly decreased the percentages of hemolysis of vitamin E-deficient chick and kid erythrocytes, whereas the addition of albumin or glucose showed no protective effect. The addition of superoxide dismutase alone showed no such effect (data not shown). The addition of manganous or cobaltous ions and DTT showed protective effects against the Tween 20 hemolysis in both species.
The effect of catalase suggests that the hemolyzing action of Tween 20 might be mediated through hydrogen peroxide. However, according to previous work[3,7], hydrogen peroxide per se is not the hemolyzing agent and catalase may react directly with some intermediate compound which is the actual hemolysing agent formed during the oxidation process. Hydrogen peroxide or dialuric acid-induced hemolysis is accompanied by membrane lipid peroxidation[8,9]. Manganous and cobaltous ions become inhibitors of lipid peroxidation in vitamin E-deficient erythrocytes[10], in vitamin E-deficient microsomes[11] and in normal mitochondria[12]. DTT is a potent inhibitor of lipid peroxidation in the thalassemic erythrocytes[13]. Tween 20 may cause oxidative damage to membrane lipid through the formation of peroxides and free radicals as suggested before[14,15]. Considering the species susceptibility to the Tween 20 hemolysis[1,2], the species peculiarity of membrane lipid-protein complexes[16] must be important as well as the sizes of hydrophilic and hydrophobic moieties of the surfactant[17]. Vitamin E plays a structural role in protecting membrane lipid-protein complexes against the oxidative damage caused by Tween 20. Although the precise mechanism of Tween 20 hemolysis is not known, this work strongly suggests that the hemolysis caused by Tween 20 may have a similar origin to that induced by hydrogen peroxide or dialuric acid.

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Regulatory properties of 14-day embryo and adult hen heart AMP-deaminase; the influence of pH on the enzyme activity

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Summary. The variation of kinetic parameters with pH for the reaction catalysed by the purified 14-day embryo and adult hen heart AMP-deaminase was shown to be similar but not identical. The pH-dependence of the half-saturation constant ($K_{0.5}$) is well pronounced, and the plot of $pK_{0.5}$ vs pH is manifested as a bell-shaped curve for both developmental forms of the enzyme. In contrast to that, the maximum velocity of the reaction (V_{max}) catalyzed by these enzymes does not change significantly in the range pH 5.6–7.4.

Heart muscle AMP-deaminase of vertebrate animals has been the subject of detailed study in our laboratory for several years. Similarly to the skeletal muscle enzyme, heart AMP-deaminase is a regulatory enzyme the activity of which is modulated by several low-molecular-weight metabolites. The most important allosteric effectors of the enzyme are sodium ions, ATP, ADP and orthophosphate[2–5]. ATP strongly activates heart muscle AMP-deaminase, whereas inorganic phosphate significantly inhibits the enzyme. GTP and active fatty acids also take part in the regulation of heart adenylate deaminase activity[6,7].

It has been found recently that the influence of temperature on the activity of 1-day chicken heart AMP-deaminase is different from its influence on the adult hen heart enzyme[8]. More detailed study showed that there exist at least two developmental forms of chicken adenylate deaminase in the heart. The enzyme isolated from heart extract of the 14-day embryo differs from the one from adult hen with respect to chromatographic and regulatory properties[9].
In the present paper the results of a study on the influence of pH on the activity of both developmental forms of chicken heart AMP-deaminase are reported.