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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0014-4754/84/030258-02$1.50 + 0.20/0

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.

Materials and methods. White Leghorn fertilized eggs and hens were used for experiments. About 200 embryos were used for 1 preparative run.

Purification of the enzyme: AMP-deaminase from heart extracts was purified by chromatography on phosphocellulose essentially according to the procedure of Smiley et al.[10] as described previously[9]. The specific activity of the enzyme specimen was 1.1 and 2.1 µmoles of ammonia liberated per mg of protein per min at 6 mM substrate concentration for 14-day embryo and adult hen respectively.

Enzyme assay: The activity of the enzyme was assayed from the ammonia formed, determined colorimetrically with the use of the phenol-hypochlorite method[11]. Three parallel incubations were carried out. The incubation mixture in a final volume of 0.5 ml contained 0.05 M sodium-cacodylate buffer, 0.1 M KCl, 1 mM ATP and different (from 0.2 up to 10 mM) concentrations of adenylate. The pH of each experimental mixture (pH values between 5.6 and 7.4) was determined after all the compounds had been added. After equilibration of the temperature (30 °C), 20 µl of appropriately diluted enzyme was added into the medium to start the reaction, which was carried out for 10 min and terminated by the addition of phenol-hypochlorite reagent.

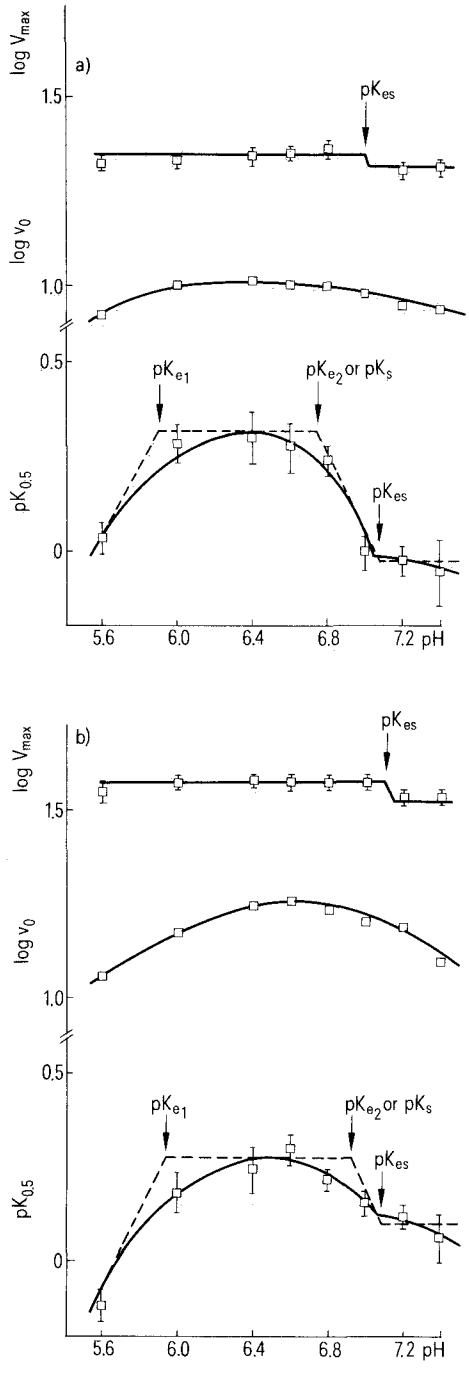
Calculation of kinetic parameters: The statistical method of Wilkinson[12] was used to calculate the substrate concentration giving half-maximum velocity ($K_{0.5}$) and maximum velocity of the reaction (V_{max}).

Results and discussion. There are several ways in which pH may influence enzyme activity. In general, pH effects are due to change in the state of ionization of the reaction mixture components. Either the free enzyme, the substrate or the enzyme-substrate complex may undergo such changes. At a sufficiently high, saturating concentration of the substrate, the whole of the enzyme exists in the form of the enzyme-substrate complex and the velocity of the enzyme-catalyzed reaction simply illustrates the rate of the breakdown of this complex. So, the influence of pH on the maximum velocity of the reaction is determined only by the state of the ES complex ionization. In contrast to that, the influence of pH on the substrate constant (K_s) is affected both by the state of ionization of the free enzyme and of the substrate[13,14].

The kinetic parameters calculated for the 2 forms of the enzyme show very similar characteristics of pH dependence. The value of V_{max} remained practically unchanged over the whole range of pH values tested. In contrast to that, the value of the half-saturation constant was dependent on pH in a distinct way. An abrupt decrease in the acidic region of pH was characteristic for both developmental forms of the enzyme. Some differences were also seen when pH was further increased.

To determine the ionization constants (pK), the data obtained are plotted on the figure, according to the logarithmic method of Dixon[15,16]. The lower, bell-shaped curves in the figure represents the plot of negative logarithms of $K_{0.5}$ values against pH. The plot starts at lower pH values as a line with slope +1, transforming after a discontinuity at about pH 5.9 into a zero slope line. At pH 6.8 (for the embryo enzyme) or 6.9 (for the hen enzyme) the second discontinuity in this plot may be observed and the zero slope line transforms into a line with a slope −1. The next bend of the line, creating discontinuity at a pH of about 7.1 is more distinct for the embryo heart AMP-deaminase. According to Dixon's rules for pH-induced effects[13], each bend in the pK_m plot represents the change in the ionization state of the free enzyme or (and) free substrate. The change in the ionization state of the enzyme-substrate complex, which may be described by the discontinuity in the log V_{max} plot probably occurs both in the case of embryo and of hen heart deaminase. The discontinuity of the upper line of the plot observed at a pH of about 7 supports this possibility.

The middle curves in the figure represent the change of the initial velocity of the reaction catalyzed by embryo and hen heart AMP-deaminase at a relatively low (0.4 mM) concentration of the substrate. The difference in the shapes of the 2 curves is remarkable. The curve of the log v_0 versus pH



The effect of pH on $K_{0.5}$, V_{max} and v_0 of the reaction catalyzed by AMP-deaminase isolated from heart muscle of 14-day embryo (a) and adult hen (b). The arrows indicate the supposed ionization constants of the free enzyme (pK_e), the enzyme-substrate complex (pK_{es}) and of the substrate or the activator (pK_s). For experimental conditions see text.

for adult hen heart enzyme has a distinctly arc-shaped profile, which means that deaminase from this tissue is much more affected by the pH under these conditions.

The ionization constant values of the substrate (pK for AMP: 6.1-6.4) and the activator (pK for ATP: 6.5-6.9) lie in the region of the changes observed. Thus the bend at the acidic side of the pH scale (fig.) may correspond to the pK for the free enzyme. The $pK_{0.5}$ plot concaved downward at the neutral pH agrees well with the discontinuity of the curve in the log V_{max} plot and probably exhibits the change in the ionization state of the enzyme-substrate complex.

It has been shown previously[9], that the presence of ATP (even at low concentration) transforms the sigmoid-shaped kinetics of the chicken heart AMP-deaminase into a hyperbolic one. It is evident from the experimental data obtained in this work that in the presence of 1 mM ATP both developmental forms of the heart enzyme showed hyperbolic kinetics throughout the whole pH-range tested. Thus the half-saturation constant ($K_{0.5}$) has the significance of the Michaelis constant (K_m) in these conditions.

The most interesting feature of the data presented in this paper seems to be the fact that the maximum velocity of the reaction, but not the half-saturation constant was practically pH-independent over the whole pH-range tested. This suggests that the chicken heart AMP-deaminase is influenced by hydrogen ions according to the unireactant model described by Michaelis and Davidsohn[17]:

$$\begin{array}{ccccccc} & & E & & & & E \\ K_1 & & \Updownarrow & & & & \Updownarrow \quad K_i \\ & EH + S & & \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} & EHS & \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} & EH + P \\ K_2 & & \Updownarrow & & & & \Updownarrow \quad K_2 \\ & & EH_2 & & & & EH_2 \end{array}$$

where K_1 and K_2 are the equilibrium constants referring to the first and the second stages of ionization of the enzyme molecule.

The rate equation for this model has the form:

$$v_0 = \frac{V_{max}}{1 + \frac{K_m}{[S]}\left(1 + \frac{[H]}{K_1} + \frac{[H]}{K_2}\right)}$$

where $V_{max} = k_{+2}[E_0]$.

It is noticeable from this equation that the expression for K_m is pH-dependent, but that for V_{max} it is not.

The above comparison of the effect of pH on the reaction catalyzed by adenylate deaminase from the heart of 14-day chicken embryo and of adult hen confirms the notion[9] that also in this respect the enzyme is not the same at these 2 ontogenetic stages.

1 Acknowledgments. The author wishes to thank Prof. M. Żydowo for interest in this work. This work was supported by a grant from the Ministry of Science, Higher Education and Technology within the project R.I.9, 01.06.

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0014-4754/84/030259-03$1.50 + 0.20/0

Hypolipidemic effects of garlic oil in rats fed ethanol and a high lipid diet

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Summary. Feeding of ethanol and a high fat-high cholesterol diet to rats markedly increased the total lipids in the liver, and cholesterol and triglyceride levels in the serum, liver and kidneys. However, when ethanol mixed with 0.5% garlic oil was fed to animals maintained on the high fat-high cholesterol diet, these lipid levels were significantly reduced to levels near to those seen in untreated control rats. Garlic oil did not reduce the serum albumin or the total proteins of liver, kidneys or serum when fed along with ethanol. Probably the garlic oil enhances the catabolism of dietary cholesterol and fatty acids.

Ethanol administration is known to enhance the endogenous syntheses of cholesterol[2] and fatty acids[3-5], which accumulate in the liver. Ethanol also decreases the catabolism of dietary cholesterol[6,7] and fatty acids[8,9]. Alcoholic hyperlipemia and steatosis are potentiated by a high lipid diet[5,10,11].

Massive supplementation of the diet with choline in human volunteers[12] or experimental animals[13,14] failed to prevent the alcohol-induced fatty liver. High protein diet[15] or a diet containing a complete mixture of amino acids or casein, methionine and cysteine also was ineffective in preventing alcoholic fatty liver[16-18]. Garlic oil is a potent hypolipidemic agent in sucrose-fed animals[19] and in animals fed ethanol and a low lipid diet[20].

Garlic extracts fed to normal rats were found to depress the lipid levels in liver and blood[21,22]. Hence the present work