enzymic activity of ATPase stimulated by Mg^{2+} or Ca^{2+}. As seen in the right-hand panel in figure 1, mitochondrial ATPase inhibitor, (F_1-inhibitor) also inhibited the enzymic activity of Mg^{2+}-stimulated ATPase. The inhibitory action was enhanced with increasing concentrations of F_1-inhibitor and remained constant (40%) at a concentration of more than 20 µg F_1-inhibitor. No complete loss of the ATPase activity with F_1-inhibitor occurred; the reason for this is not clear.

Component TN-I was digested with trypsin and the inhibitory effect of the digested component TN-I on the enzymic activity of Mg^{2+}-stimulated ATPase was tested. As seen in figure 2, after a 2-min-digestion of component TN-I with trypsin, the inhibitory action was not observed and the ATPase activity was completely restored. However, the actomyosin ATPase activity was inhibited by the digested component TN-I. This is the same result as that reported by Shigekawa and Tonomura[16]. The disc-electrophoretic pattern of the digested component TN-I had no band corresponding to that of native component TN-I and had bands with smaller molecular weights than that of component TN-I. This indicates the significance of the conformation of the component TN-I molecule for its inhibitory action on partially purified *E. coli* ATPase. It is well known that one of the troponin components, TN-C, which has binding-ability for component TN-I, reverses the inhibitory action of the component TN-I on the enzymic activity of actomyosin ATPase. When component TN-C (34.8 µg) was added to the reaction mixture containing the partially purified *E. coli* ATPase (2.3 µg) and component TN-I (31.7 µg), no inhibition of the ATPase activity was observed. This shows that binding of component TN-C to component TN-I causes the loss of the inhibitory ability of component TN-I on the partially purified *E. coli* ATPase.

We have reported so far the inhibitory action caused by the interaction between ATPase and their inhibitors from different sources[8-11]. Component TN-I inhibits mitochondrial, chloroplast and bacterial ATPases, and F_1-inhibitor inhibits muscle, chloroplast and bacterial ATPases. For the muscle, mitochondrial and chloroplast ATPases, the ATPase activities were inhibited by component TN-I and also restored by component TN-C as reported previously[17,18]. These ATPases, including the bacterial ATPase, seem to be inhibited in a similar interaction between the ATPase and component TN-I.

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Effect of the ionic strength on the kinetic properties of the mitochondrial L-malate dehydrogenase

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Summary. Increase of the ionic strength inhibits the catalytic activity of the mitochondrial MDH, reduces substrate inhibition and decreases the affinity of substrates for the enzyme.

The catalytic activity of the mitochondrial L-malate dehydrogenase (MDH) is strongly affected by changes in the ionic environment, while the cytoplasmic MDH is insensible to such changes[3]. It was shown[3] that nature and extent of the ionic strength effect on the mitochondrial MDH depend on the oxaloacetate concentration. The mitochondrial MDH shows substrate inhibition[4] at high oxaloacetate (or NADH) concentrations, and one would expect that this concentration dependence of the ionic strength action reflects mainly a kinetical effect. It is the purpose of this study to investigate this kinetical effect and to examine the ionic strength action on the kinetical parameters of the enzyme.

Materials and methods. The mitochondrial L-malate dehydrogenase (EC 1.1.1.37) from beef-heart was purified according to the procedure described by Englard and Siegel[5]. Mitochondria were prepared according to the methodology of Crane et al.[6]. The purified enzyme was stored at low temperature and appropriately diluted with tris-acetate buffer (pH 8.0 and 0.05 M in acetate) at the moment of use. The experimental data were obtained by monitoring the NADH (reduced nicotinamide adenine dinucleotide) oxidation at 340 nm during the first min of reaction. The minimal ionic strength was 0.05 M and can be attributed to the components of the buffer (tris-acetate, 0.05 M in acetate). Further increments in the ionic strength were obtained by the addition of several strong electrolytes, but all data presented in this paper were obtained in the presence of KCl. The experimental data presented in this paper are the average values of 3 independent estimates.

Statistical treatment of the data (least-squares fits) were made according to Wilkinson[7].

Results and discussion. The effect of several neutral salts was investigated with similar results. The effect is independent of the time of incubation and reversible (experiments not shown). Figure 1A shows the combined effect of ionic strength and oxaloacetate concentration changes. The ionic strength effect at low substrate concentrations is inhibitory. The maximal velocity is decreased and the optimum concentration is shifted toward higher concentrations leading to a reduction in the relative degree of substrate inhibition. The oxaloacetate concentration dependence may be described very well by the relation[4,8]:

$$v = \frac{V_{ap}[oxal]}{K_{ap} + [oxal] + [oxal]^2/K_{iap}} \quad (1)$$

v is the reaction velocity, V_{ap} the apparent maximal velocity (function of the NADH concentration), K_{ap} the apparent Michaelis constant for oxaloacetate and K_{iap} the apparent substrate inhibition constant for oxaloacetate. The values for K_{iap} at ionic strengths 0.05, 0.2 and 0.4 M are 1.3, 1.7 and 3.9 mM respectively, denoting an absolute reduction of the substrate inhibition.

The combined effect of ionic strength and NADH concentration changes, which may be seen in figure 1B, shows similar aspects. It should be noted, however, that a more pronounced absolute reduction of the substrate inhibition occurs at high ionic strength. The NADH substrate inhibition takes place at relative low NADH concentrations and seems to have a more complicated mechanism than the oxaloacetate substrate inhibition[4]. Tentatives to fit expressions like equation (1), and more complex ones but with some theoretical meaning failed[8] and consequently no numerical parameter relating substrate inhibition by NADH and ionic strength changes could be obtained from the data.

The mitochondrial MDH shows Michaelis-Menten kinetics at low substrate concentrations and an ordered bi-bi reaction mechanism has been proposed for the enzyme[9]. Equation (2) relates, under these conditions, the reaction velocity to the concentrations of both, oxaloacetate and NADH:

$$v = \frac{V_{max}[oxal][NADH]}{K_{iNADH}K_{oxal} + K_{oxal}[NADH] + K_{NADH}[oxal] + [oxal][NADH]} \quad (2)$$

V_{max} is the maximal reaction velocity, K_{oxal} and K_{NADH} are the Michaelis constants for oxaloacetate and NADH respectively. K_{iNADH} is the dissociation constant of the enzyme-NADH complex. This equation may be used to compute the kinetic parameters for both substrates under conditions where substrate inhibition may be neglected[4,8]. The results are shown in figure 2. V_{max} is reduced and both Michaelis constants increase several times when the ionic strength is raised from 0.05 to 0.4 M. K_{iNADH}, however, increases only to a small extent (approximately 60%). V_{max}, K_{oxal} and K_{NADH} are functions of several individual velocity constants[8] and if one assumes that the most of them may be affected in some particular way by the ionic strength, then the global effect will be an inextricable combination of several effects. K_{iNADH}, however, a dissociation constant, should be more accessible to theoretical analysis. It has been shown that dissociation constants of ionic species increase when the ionic strength of the medium is increased[10]. The direction and the extent of this change are consistent with the Debye-Hückel equation, which means that it may be attributed to changes in the activity coefficients[10]. Calculations, based on theoretical and experimental data[10,11] predict a 55% increase in the value of K_{iNADH}, when the ionic strength is raised from 0.05 to 0.4 M. The

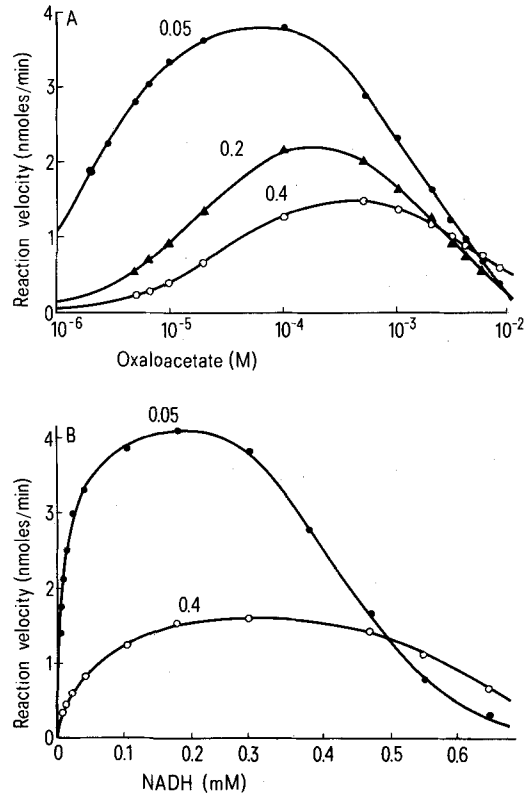

Fig. 1. Reaction velocity vs substrate concentration at several ionic strengths. Data were obtained at 37 °C in tris-acetate buffer (pH 8.0) 0.05 M in acetate. The ionic strengths are indicated on each curve. Increase in ionic strength was obtained by addition of KCl. *A* Oxaloacetate concentration dependence. The fixed NADH concentration was 100 µM. The traced curve represents a least-squares fit of equation (1) to the data. *B* NADH concentration dependence. The fixed oxaloacetate concentration was 100 µM.

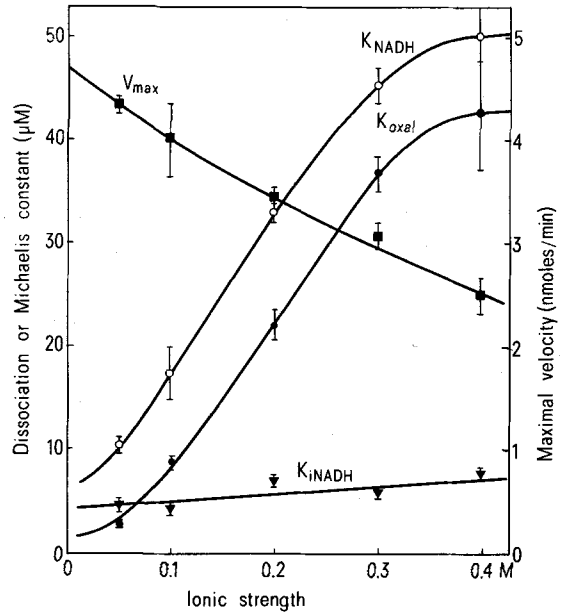

Fig. 2. Variation of the kinetic constants with ionic strength. Data were obtained at 37 °C in tris-acetate buffer (pH 8.0) 0.05 M in acetate. The nature of each constant is indicated on the corresponding curve. The reaction velocity was measured at several oxaloacetate (1-50 µM) and NADH (5-100 µM) concentrations, and the kinetic constants were computed by fitting equation (2) to the experimental data. Bars represent SE.

increase actually observed (60%) agrees fairly well with the theoretically expected change. The reduction of the substrate inhibition observed on figure 1 is also expected from the Debye-Hückel theory which predicts an increase in the value of the substrate inhibition constants[12]. The true substrate inhibition constants, however, cannot be measured, because the mechanism of the substrate inhibition is unknown (NADH) or not very clear (oxaloacetate). Consequently, in this case, no comparison between theory and experiment can be made.

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Reduction in dietary vitamin E prevents onset of hypertension in developing spontaneously hypertensive rats[1]

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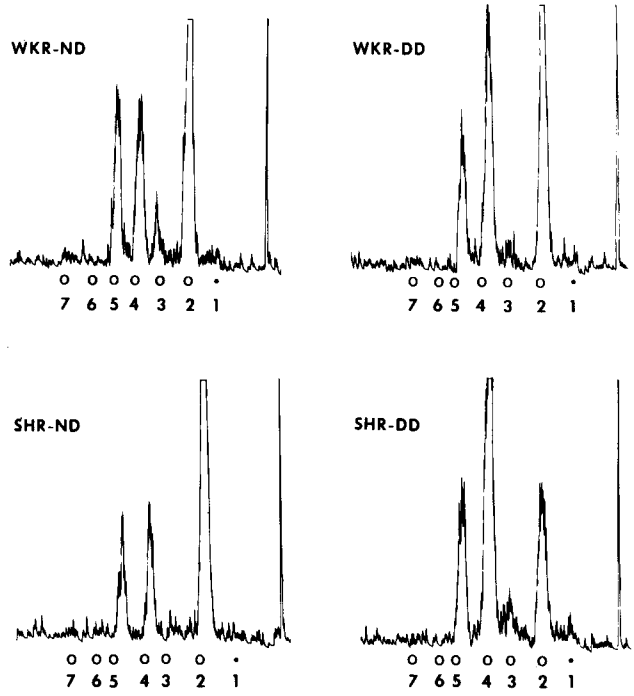
Summary. Reduction in dietary vitamin E intake in developing spontaneously hypertensive rats abolished the onset of hypertension which is normally evident by 3 months of age.

The spontaneously hypertensive rat is a popular model for the study of human essential hypertension. Because the rats are not hypertensive at birth they provide a convenient model for studies on the etiology of hypertension since they can be studied in various stages of hypertension, i.e., pre, early, and established stage.

In previous studies we demonstrated an age-dependant increase in renal prostaglandin catabolism in the normal rat which reaches a peak at 19 days postnatally (60-fold relative to the adult) thereafter quickly dropping to adult levels by day 40. Prostaglandin biosynthetic activity did not change[2]. The potential importance of this prostaglandin catabolic activity profile was quickly realized when a similar study was carried out in the developing spontaneously hypertensive rat. While renal prostaglandin catabolism in these rats was normal at birth, it quickly reached levels that were 2–2.5-fold deficient over normal by 19 days postnatally which preceded elevations in systemic blood pressure[3]. These experiments suggested a possible correlation between deficient prostaglandin catabolism during a 'critical' postnatal stage in renal development and the subsequent onset in elevated blood pressure in the spontaneously hypertensive rat.

In our attempts to alter prostaglandin catabolism in the normal and the spontaneously hypertensive rat, we tested variations in dietary intake in vitamin E which as an antioxidant could participate in prostaglandin biosynthetic or catabolic pathways. As large amounts of this vitamin are known to inhibit prostaglandin biosynthesis[4, 5], we set out to investigate the effect of a reduction in its dietary intake.

Materials and methods. Pregnant rats (Wistar, Aoki-Okamoto spontaneously hypertensive and Kyoto normotensives) were purchased from Taconic Farms, Germantown, New York. Directly upon giving birth, litters were divided into 4 groups (2 litters/group) and placed on diets containing normal content of vitamin E (132 IU/kg - Purina) or deficient in vitamin E (12 IU/kg - Teklad). At 21 days, rats were divided and placed 2 in each cage and maintained on



Radiothin layer profiles showing enhanced prostaglandin catabolism in renal cortical homogenates from hypertensive rats (SHR) maintained on a vitamin E deficient (12 IU/kg) diet (DD). Also shown for comparison are parallel incubations of homogenates of renal cortex from normotensive rats (WKR) fed normal diet (ND, vitamin E = 132 IU/kg) and DD and SHRs fed ND. The assay was performed using 0.5 ml homogenate (1/10 w/v) in 0.05 M KH_2PO_4-NaOH buffer (pH 7.4), in 2 tubes containing 9 β-$^3H_1$-$PGF_{1\alpha}$ (NEN, 200,000 cpm) and either 1 or 5 µg $PGF_{1\alpha}$ (Upjohn Co. Kalamazoo) and NAD^+ (4 mM, Sigma). After 10 min at 37 °C incubations were terminated with 5 vol. ethanol. The scans refer to experiments using 1 µg substrate $PGF_{1\alpha}$.