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Influence of Vit. E Deficiency in Rabbit on Urinary Excretion of Free Amino Acids.* (22355)

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It has been shown that urinary excretion of free amino acids is elevated in patients with progressive muscular dystrophy(1). Also it has been reported that vit. E-deficient rabbits excrete extra quantities of free amino acids in urine although no actual data have been presented(2,3). In view of these considerations, and since any information concerning metabolic disturbances in vit. E deficiency may aid in elucidation of the biochemical role of the vitamin, we have determined the influence of vit. E deficiency on urinary excretion of amino acids by rabbits.

Methods. White New Zealand rabbits, weighing initially about 500 g each, were given the same purified diet previously described(4) except that folic acid was omitted. Controls received this diet with oral supplements of 10 mg of alpha tocopherol acetate 2 times per week. There were 4 rabbits in each group. The animals were kept in individual metabolism cages and daily urine samples were collected throughout the experiment. Urine samples were analyzed for free amino acids by the copper method(5). The experiment was terminated when unsupplemented animals exhibited early stages of muscular dystrophy.

Results. Vit. E deficiency led to a considerable increase in excretion of free amino acids in urine. At the time of appearance of earliest physical signs of muscular dystrophy urinary excretion of free amino acids was tripled (Table I). Actually there was a significant increase in excretion of amino acids before any physical signs of dystrophy were apparent. This agrees with the observation of Ames (2).

TABLE I. Influence of Vit. E on Average Daily Urinary Excretion of Free Amino Acids by Rabbits.

Days on diet	mg amino N/kilo body wt	
	Without vit. E	With vit. E
1- 5	6.6	5.2
6-11	5.9	4.5
11-15	4.5	4.3
16-20	5.7	3.7
21-25	8.6	3.3
26-31	13.5	4.5

Increased excretion of free amino acids in vit. E deficiency may indicate an impaired incorporation of amino acids into protein, an increased breakdown of tissue proteins, or an impairment in normal metabolic disposal of amino acids. Experiments with C^{14} labeled glycine have indicated that vit. E deficiency does not significantly affect incorporation of that amino acid into protein or its oxidation to CO_2(6). This would suggest that elevated excretion of free amino acids in vit. E deficiency is due to accelerated breakdown of tissue protein, probably primarily skeletal muscle.

Summary. Vit. E deficiency in the rabbit results in increased excretion of free amino acids in urine. This increase in amino acid excretion is apparent before any physical signs of dystrophy are observed.

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Sedimentation Behavior of Desoxypentose Nucleic Acids from Normal and Pathologic Human Spleens. (22356)

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Previous work in this laboratory has demonstrated that the desoxynucleic acids (DNA) of normal and leukemic human cells differ in some physico-chemical characteristics(1,2). Interest in this study of macromolecular structures of normal and malignant cells has been greatly stimulated by the "template hypothesis"(3-5). According to this hypothesis, neoplastic growth would be the consequence of an abnormal event in the self duplication of some macromolecules in the cell(6), resulting in a malignant transformation of some cytoplasmic and/or nuclear structures.

Although the "template hypothesis" concerns primarily the role of ribonucleic acid (RNA) in the mechanism of specific protein synthesis, it could be extended to the role of DNA in mitosis and gene duplication. For this reason the sedimentation constant of DNA from normal and leukemic cells was studied.

Material and methods. DNA was prepared from calf thymus, normal human spleen removed surgically after traumatic rupture, human spleens from cases of multiple myeloma, Brill-Simmers disease, myeloid leukemia and hereditary spherocytosis. The organs were frozen with dry ice immediately after removal and powdered in a high speed mixer with additional dry ice. Thirty grams of powdered tissue were suspended in 100 ml of NaCl-Na Citrate solution (0.14 M NaCl; 0.01 M Na Citrate) and homogenized for 2 minutes at 12,000 r.p.m. The material was then filtered through 3 layers of gauze, centrifuged, and the sediment washed 3 times. The sediment was then resuspended in 20 volumes of 1.7 M NaCl-0.01 M Na Citrate solution and homogenized again for 30 seconds at a lower speed. DNA sodium salt was extracted according to the method of Gulland (7) as modified by Chargaff *et al.*(8), then dialyzed against distilled water for 72 hours at 0-4°C and lyophilized. The N/P ratio as well as other chemical properties of the obtained DNA, correspond closely to the theoretical values and have been published elsewhere(2). DNA was dissolved in cacodylate buffer of ionic strength 0.3 and pH 7(9) by gentle overnight stirring in the cold room (0-4°C). The DNA concentration of the solution was calculated from its phosphorus content (P DNA) determined with the method of Fiske and Subbarow, after conversion of the arsenic from the penta- to the trivalent form(10). The samples were analyzed at room temperature within 24 hours of being dissolved. Sedimentation data were obtained in the Spinco Model E analytical ultracentrifuge. Sedimentation constants were corrected for a solvent having viscosity and density of water at 20°C; the partial specific volume of DNA was assumed to be 0.55(11,12).

Results. Typical schlieren photographs of DNA sedimentation boundaries are seen in Fig. 1. The data obtained with DNA from a series of normal and pathological spleens are shown in Fig. 2 where the S_{w20} values are plotted against the P DNA concentration of the samples. It can be seen that points fall on the same curve, regardless of the source of the DNA samples.

Discussion. The spread of values obtained with any given P DNA concentration seems to indicate a variability within each determination rather than differences in the sedimentation constant of DNA samples of dif-