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Amino Aciduria of E-Avitaminosis. Exceptional Role of Glycine.* (24734)

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An increased urinary excretion of free amino acids in Vit. E deficiency in rabbits has been reported(1,2,3,4). In those studies free amino nitrogen in urine was determined but apparently no effort was made to determine amino acid composition of the urine. It seemed desirable to determine whether this increase of free amino nitrogen was caused by uniform increase of all amino acids in urine, or if perhaps the excretion of only one or a few amino acids was elevated.

Methods. White New Zealand rabbits of mixed breed, weighing about 500 g each, were placed in metabolism cages and fed a dystrophy-producing diet *ad lib*. The diet has the same composition as that described by Young and Dinning(5) except that roughage, *i.e.* cellulose (Alphacel) was added equal to one-third of weight of the other ingredients. Water was added to form a dough which was then spread out and dried by an electric fan. The dry, hardened dough was broken into cookie-sized pieces. Three rabbits received this diet alone, 3 other rabbits received the same diet plus oral supplements of 4 mg of

α -tocopherol acetate/kg body weight twice weekly. Daily urine samples were collected, stored under toluene at 4°C, and analyzed for amino acid composition by 2-dimensional paper chromatography, for glycine by the method of Alexander *et al.*(6), and for amino-nitrogen by the copper method(7). Creatine and creatinine were determined by the Folin method in urine samples collected during first and fourth week of experiment. When the Vit. E-deficient animals developed severe muscular weakness and exhibited a urinary creatine:creatinine ratio of over 2 after about 4 weeks feeding, they were sacrificed for purposes not related with the present report. Control animals were sacrificed at exactly 4 weeks.

Results. The 2-dimensional paper chromatograms of urine samples from deficient rabbits, showed a rather uniform pattern of ninhydrin-positive spots throughout experiment. There was no strong increase in size and color intensities of any one spot, but rather a slight uniform increase of all spots with one exception; glycine seemed to decrease, or at least there was no increase in size and color intensity of the glycine spot.

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TABLE I. Average Daily Urinary Excretion of Free Glycine and Free Amino Nitrogen by Control and Vit. E Deficient Rabbits. (Results expressed as mg/kg body wt $\pm$ stand. error.)

Period of exp. (wk)	Deficient animals			Control animals		
	Glycine	Amino N	Glycine N as % of amino N	Glycine	Amino N	Glycine N as % of amino N
1st	19.3 $\pm$ 1.5	8.4 $\pm$ 3.1	43	26.4 $\pm$ 3.4	10.9 $\pm$ 1.4	45
2nd	23.5 $\pm$ 4.3	8.9 $\pm$.3	49	25.8 $\pm$ 2.1	9.5 $\pm$ 2.0	51
3rd	19.5 $\pm$ 2.1	8.6 $\pm$ 2.4	42	25.8 $\pm$ 3.8	11.8 $\pm$ 1.0	41
4th	16.9 $\pm$ 3.2	15.5 $\pm$ 3.7	20	17.7 $\pm$.8	7.4 $\pm$ 1.0	45

Results of quantitative determinations of glycine and aminonitrogen are summarized in Table I. The slight decrease of urinary glycine in the deficient group is inconclusive in view of a decrease of glycine in the control group. But the increase of amino-nitrogen in the deficient group is certainly not paralleled by an increase of glycine. This becomes clearer from columns 4 and 7 of Table I, where glycine-nitrogen (calculated from the glycine content) is expressed as percent of total free amino-nitrogen content. In the control group glycine accounts for 41 to 51% of amino-nitrogen, glycine content in the deficient drops to 20% of amino-nitrogen.

The present results seem of interest in view of findings of other authors that glycine is in some way implicated in the dystrophic state. Tallan(8) reported increased concentration of most free amino acids in muscle extracts of dystrophic rabbits except for glycine, which was significantly reduced. This was confirmed by Smith and Nelson(9). Smith and Nehorayan(10) showed recently that reduction of tissue phosphatase observed in dystrophic rabbits is prevented by addition of 1% glycine to the diet.

Summary. 1. That excretion of free amino nitrogen in urine is elevated in dystrophic state of rabbits on Vit. E-free diet has been confirmed. 2. Glycine shows an exceptional behavior insofar as increased amino nitrogen excretion is not paralleled by increased glycine excretion.

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Autoradiography of the Isolated Nephron.* (24735)

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Detection of radioactive substances in tis-

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ues by autoradiography has been widely used. Difficulty has sometimes been experienced in interpreting the exact site of activity within the kidney owing to its complex anatomy and that histological sections do not give