Effect of Vit. E-Deficiency on Free Amino Acids of Various Rabbit Tissues.* (23038)

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Roderuck(1) demonstrated that the glutamine content of skeletal muscle of dystrophic guinea pigs was reduced from that of muscle of control animals, whereas the content of non-glutamine amino acids was unchanged. Vit. E-deficient rabbits showed less marked differences. Tallan(2) reported that in severe dystrophic rabbit, the levels of most free amino acids in muscle extracts were elevated except for concentration of glycine which was markedly reduced, even in early stages of the disease. In contrast to this finding, Dinning *et al.*(3) were unable to show any consistent effect of vit. E-deficiency upon concentration of free glycine of muscle or liver. It seemed, therefore, desirable to resolve these differences as well as to determine what effect, if any, the dystrophic condition had on concentration of free amino acids of tissue other than muscle. Since it may be presumed that free amino acids constitute a major source of the protein building blocks of tissue, the concentration of free amino acids of atrophied muscle, as well as that of supposedly unaltered organ tissue from a dystrophied animal, should be of considerable interest. The studies here reported describe the concentration of 9 free amino acids in liver, kidney, heart, spleen, brain, and muscle of normal and vit. E-deficient rabbits.

Methods. Four male rabbits weighing 1250 g to 1475 g were fed the dystrophy producing diet of Goettsch and Pappenheimer(4) while 2 litter mates consumed an adequate diet. Creatine was determined by the method of Folin(5) and it was noted that those animals on the deficient diet exhibited a slight creatinuria within 3 weeks, while controls showed none. The onset of dystrophy was considered to have started when creatine excretion rose rapidly, consequently when this point was reached, the 6 animals were sacrificed. Two animals showed a severe dystrophy as evidenced by respiratory difficulty, and inability to right themselves after being placed on their sides. The other 2 deficient animals had less pronounced outward symptoms as well as lower degree of creatinuria. Thus these two sets of deficient animals would compare with early and severe dystrophy animals described by Tallan(2). As soon as animals were sacrificed, samples of tissue were immediately homogenized with a Teflon pestle in distilled water. Aliquots of the homogenate were removed to determine dry weight of tissue, and the remainder was treated with 10% trichloroacetic acid to remove the proteins. After removal of acid with ether, the resulting solution was concentrated *in vacuo*. Quantitative determination of the amino acids was then made by Dowex-50 chromatography as previously described(6).

Results. Distribution of free amino acids in various tissues of normal and dystrophic rabbits is shown in Table I. The values are expressed as mM/100 g dry tissue, and represent the average of tissues from 2 rabbits, except where noted. The results listed in Table I show that muscle and heart of severely dystrophic animals had a general increase in concentration of free amino acids with the exception of taurine and glycine. The same results were also evident in mildly dystrophic animals although the differences were not as pronounced. On the contrary, Table I demonstrates that brain homogenates of severely dystrophic animals exhibited a general decrease in concentration of free amino acids, especially of serine. Only ethanolamine phosphate and glutamic acid displayed a slight increase. This same trend was also evident in the brain of mildly dystrophic animals. Kidney homogenate of dystrophic animals likewise evinced a decrease in certain amino acids, *viz*: ethanolamine phosphate, serine,

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TABLE I. Free Amino Acid Concentrations of Tissues from Normal and Vit. E-Deficient Rabbits. Values expressed as mM/100 g dry tissue and probable error of the mean.

Amino acid	Muscle		Heart		Brain		Kidney		Spleen		Liver	
	Normal	Vit. E-def.	Normal	Vit. E-def.	Normal	Vit. E-def.	Normal	Vit. E-def.	Normal	Vit. E-def.	Normal	Vit. E-def.
Ethanolamine phosphate	.13 ± .04	.31 ± .03	.39 ± .02	.41 ± .03	1.21 ± .08	1.43 ± .09	2.92 ±1.00	1.90 ± .03	2.98 ± .10	2.80 ± .41	.35 ± .06	.81 ±.06
Taurine	.74 ± .12	.43 ± .19	6.72 ±1.4	6.6 ±1.4	1.08 ± .04	1.04 ± .02	1.51 ± .39	1.75 ± .03	2.74 ± .14	2.83 ± .24	.24 ± .05	.63 ±.24
Aspartic acid	.10 ± .00	.19 ± .04	.53 ± .09	.56 ± .15	1.96 ± .24	1.53 ± .08	.65 ± .37	.70 ± .03	1.22 ± .05	1.17 ± .07	.72 ± .28	.76 ±.06
Threonine	.11 ± .01	.32 ± .06	.11 ± .01	.20 ± .02	.23 ± .00	.18 ± .01	.21 ± .13	.46 ± .01	.44 ± .03	.57 ± .05	.16 ± .00	.15 ±.00
Serine	.48 ± .07	.79 ± .20	.46 ± .04	.72 ± .18	2.32 ± .60	.93 ± .12	1.74*	.90 ± .02	1.14 ± .11	1.19 ± .06	.75 ± .06	.45 ±.07
Glutamic acid	.56 ± .12	.67 ± .08	1.30 ± .06	1.44 ± .11	3.54 ± .43	4.05 ± .78	2.94*	2.47 ± .06	3.73 ± .15	4.02 ± .13	1.29*	1.41 ±.27
Proline	.00 ± .00	.98 ± .15	.32 ± .00	.00 ± .00	.00 ± .00	.00 ± .00	2.04*	1.73 ± .04	1.21 ± .20	1.56 ± .11	.60*	.81 ±.10
Glycine	1.77 ± .16	1.04 ± .13	.65 ± .09	.34 ± .05	.86 ± .03	.72 ± .05	4.58*	1.96 ± .03	2.61 ± .16	1.83 ± .08	1.50*	1.77 ±.02
Alanine	.66 ± .16	1.10 ± .22	1.86 ± .04	2.31 ± .30	.51 ± .00	.46 ± .05	1.58*	1.13 ± .01	.94 ± .04	1.26 ± .01	1.28*	.93 ±.09

* Single determination.

alanine, and glycine. Only threonine showed a distinct increase. Table I indicates that spleen homogenate showed a general increase in concentration of most amino acids, only glycine exhibited a significant decrease. Liver homogenate manifested an increase in ethanolamine phosphate and taurine, with only alanine and serine showing pronounced decreases.

Discussion. Perhaps the most important point to be made from these experiments is that vit. E-deficiency in rabbits led to a marked reduction in free glycine in 5 of the 6 tissues examined. Only liver showed a slight increase in glycine. Tallan found similar results in dystrophic muscle, *viz.* a decrease in free glycine of muscle approximating 46% as compared to our value of 42%. These results therefore differ from Dinning *et al.*(3) who demonstrated a slight increase in glycine of vit. E-deficient rabbit muscle. Serine, a known precursor of glycine, was markedly reduced in the kidney, liver, and especially the brain.

It has been shown(7) that there is an increased turnover rate of nucleic acids in vit. E-deficient animals. Dinning *et al.*(3) have also demonstrated that vit. E-deficiency led to greatly increased incorporation of formate-C^{14} into nucleic acid purines, but resulted in a decreased incorporation of glycine-(1)-C^{14} into purines. These results were explained on the basis of an exchange reaction of the 2 position of the purine ring without complete degradation of the molecule. Inasmuch as only the alpha carbon of glycine has been shown to be a precursor of formyl fragments, the work of Dinning *et al.*(3) does not exclude the possibility that glycine is contributing its methylene carbon to the 2 position of the purine ring since the C^{14} of their injected glycine molecule was not located in the methylene portion.

Our data suggest that perhaps glycine, as well as its precursor serine, may be acting as the main source of the formyl fragment. This would tend to deplete these two amino acids from the various metabolic pools. The possibility also exists that there is an accelerated rate of protein breakdown to furnish the

needed glycine. It is of interest that Weinstock *et al.*(8) have recently shown proteolytic activity of muscle from vit. E-deficient rabbits to be increased. This could account for the observed increase in other free amino acids of dystrophic muscle.

On the basis of these observations, it is concluded that vit. E-deficiency in rabbits is characterized by a distinct pattern change in the free amino acid levels of various tissues. The decrease in glycine suggests that it is in some unknown way implicated in the dystrophic state.

Summary. 1. Concentration of free amino acids from 6 tissues of normal and vit. E-deficient rabbits has been determined by Dowex-50 chromatography. 2. There was a general increase in free amino acids of muscle, heart, spleen, and liver of dystrophic animals. Only glycine and serine were markedly reduced. 3. Kidney and especially brain showed a general decrease in free amino acids. 4. Concentration of glycine was more consistently reduced in various tissues than other amino acids.

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Comparative Androgenic and Anabolic Effects of Several Steroids. (23039)

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The common androgens, testosterone propionate and methyl testosterone, produce anabolic as well as androgenic effects(1,2). In man, the androgenic activity frequently limits the use of these compounds as anabolic agents. Attempts to increase relative anabolic potency have led to development of methyl androstenediol(3) (Methostan, Methandriol), androstanolone(4) (Neodrol, Stanlone) and 17 - ethyl - 19 - nortestosterone(5) (Nilevar). The present study was underaken to compare the anabolic and androgenic potencies of these newer agents with testosterone propionate, methyl testosterone and unesterified testosterone.

Materials and methods. The method used was that of Eisenberg and Gordan(6). The rats were castrated at 23-25 days of age. Starting 3 weeks later, the compounds dissolved in corn oil, were administered intramuscularly daily for 7 days. Testosterone, testosterone propionate and methyl testosterone were compared with methyl androstenediol, androstanolone and 17-ethyl-19-nortestosterone. Eight rats were used at each dose level and total doses of 0.02 to 5 mg per rat were administered. A total of 432 rats was used; of these, 88 served as oil-treated controls. Gains in body weight, as well as terminal weights of the seminal vesicle and ventral prostate glands and levator ani muscles, were examined and compared with these parameters in controls autopsied at the same time. The regression lines shown in Fig. 1 were calculated by the method of least squares using only those values at and above the minimal effective range.

Results. Anabolic effects. Increase in weight of the levator ani muscle was used as a measure of anabolic activity. In Fig. 1, the differences between weight of this muscle in the treated animals and in controls run at the same time are plotted against the log of the dose. Marked anabolic responses were