Creatine Metabolism in Vitamin E Deficiency.* (23660)

JAMES S. DINNING† AND COY D. FITCH
Department of Biochemistry, School of Medicine, University of Arkansas, Little Rock

It is well established that creatine metabolism is deranged in vit. E-deficient animals. The general syndrome is characterized by an increased concentration of liver creatine, an increased urinary excretion of creatine and by a decreased concentration of creatine in skeletal muscle. This condition may be the result of an impaired incorporation of creatine into skeletal muscle of vit. E-deficient animals, thus leading to an increased creatinuria, or alternatively creatine may be poorly retained in the muscle, thus resulting in a rapid turnover of skeletal muscle creatine and its subsequent excretion in the urine. Data submitted in previous reports have supported the latter alternative(1,2). The present experiments were designed to measure directly the influence of Vit. E deficiency on the turnover rate of liver creatine and muscle creatine in rabbits.

Methods. The preparation of diets, Vit. E supplementation and general handling of the rabbits was the same as previously described (3). When the rabbits receiving the unsupplemented diet developed the usual deficiency signs they were selected for experiment. Four normal and 4 Vit. E-deficient rabbits were each injected intraperitoneally with 100 μc of glycine-1-C^{14} (specific activity 0.58 mc per mM) per kilo of body weight. One normal and one deficient animal were killed 1.5, 3, 4, and 6 hours after the injections. The concentrations and specific activities of kidney glycocyamine, liver creatine, skeletal muscle creatine, and heart creatine were determined. Approximately 10 g of kidney were homogenized with an equal volume of water. Trichloroacetic acid (TCA) was added to a final concentration of 5% and the protein was removed by centrifugation. One milliliter of the supernatant solution was taken for glycocyamine determination by the permutit procedure(4). Carrier glycocyamine (100 mg) was added to 10 ml of the TCA supernatant solution and dissolved by warming. The pH was adjusted to 7 and the glycocyamine was allowed to crystallize in the ice box. The glycocyamine was recrystallized from water to constant radioactivity as assayed with an end window Geiger tube. The counts were corrected to infinite thinness. The specific activity of the glycocyamine was then multiplied by the total amount of carrier added to give the total glycocyamine counts in the 10 ml of TCA supernatant solution. Based on the glycocyamine concentration of the TCA supernatant solution before carrier addition, as determined on the separate one ml aliquot, it was possible to calculate the specific activity of the kidney glycocyamine. Muscle and liver creatine specific activities were determined after conversion to creatinine. Three grams of muscle or 10 g of liver were cut in small pieces, placed in a large test tube containing 20 ml of 2 N H_2SO_4 and heated for 3 hours in a boiling water bath. After cooling, 18 ml of 2 N NaOH and 5 ml of 10% sodium tungstate were added to the tubes. The contents were filtered into a heavy duty test tube containing approximately 200 mg of Lloyd's reagent. The filtrate and Lloyd's reagent were mixed by repeated inversion for 10 minutes and then centrifuged. The creatinine formed during the heating of the tissue in acid was now adsorbed on the Lloyd's reagent. This was transferred to a 12 ml conical centrifuge tube, washed 3 times with 2 ml portions of 0.01 N HCl and centrifuged. To the sediment were added 4 ml of 0.5 N NaOH, the contents of the tube were mixed by repeated inversion for 10 minutes and centrifuged. The sediment was re-extracted with 2 ml of 0.5 N NaOH and the supernatant fluids were combined. This fraction contained the creatinine eluted from the Lloyd's reagent and was used for quantitative determination of creatinine and for the isolation of creatinine for counting. Creatinine was deter-

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TABLE I. Incorporation of Glycine-1-C^{14} into Glycocyamine and Creatine by Normal and Vit. E-deficient Rabbits.

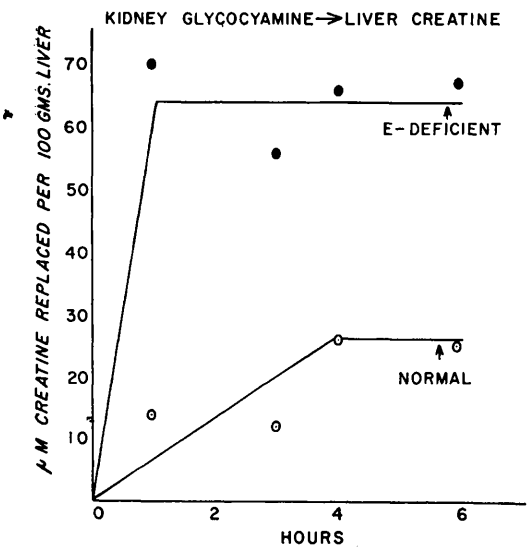
Measurements	Hr after inj.	Conc. (μM/g)		Specific activity (c.p.m./μM)	
		Normal	E-deficient	Normal	E-deficient
Kidney glycocyamine	1½	.42	.42	1050	408
	3	.64	.57	31	170
	4	.43	.39	390	171
	6	.42	.34	206	115
Liver creatine	1½	.30	1.21	424	206
	3	.18	.59	19	142
	4	.28	1.05	313	95
	6	.15	1.69	303	41
Skeletal muscle creatine	1½	37	23	.9	3.9
	3	42	24	.1	4.7
	4	32	8	2.8	10.2
	6	37	14	3.9	3.7
Heart creatine	1½	13	15	17.1	19.6
	3	11	12	1.8	14.0
	4	22	10	23.0	4.5
	6	12	21	21.9	2.5

mined by a micro procedure, in which 0.025 ml of the NaOH extract was diluted with 4 ml of water in a Klett colorimeter tube. Two milliliters of freshly prepared alkaline picrate (one part 10% NaOH and 5 parts saturated picric acid) were added and after 20 minutes the tubes were read in a Klett photoelectric colorimeter with a green filter. The creatinine concentration was calculated by comparison with a standard curve. To the remainder of the NaOH extract, after neutralization with 5 N HCL, were added 100 mg of creatinine. After the creatinine was dissolved 4 ml of 95% ethanol and 2 ml of 20% zinc chloride were added. The tubes were placed in the ice box overnight and the precipitated creatinine zinc chloride was recrystallized to constant radioactivity. The specific activity of the original creatine was calculated as described for kidney glycocyamine.

Results. The data are given in Table I. It should be noted that Vit. E deficiency resulted in an increased concentration of liver creatine, a decreased concentration of skeletal muscle creatine, and was without effect on the concentration of kidney glycocyamine. These findings are in agreement with other reports(5). Also the concentration of heart creatine was not significantly affected by Vit. E deficiency.

It is well established that in mammals glycocyamine is synthesized in the kidney and methylated to creatine in the liver. This liver creatine may then be incorporated into skeletal muscle or cardiac muscle. In order to estimate the turnover rate of liver creatine, the specific activity of liver creatine was divided by the specific activity of kidney glycocyamine from the same animal. This factor was then multiplied by the concentration of liver creatine expressed in μmoles per gram. The value so obtained was considered to represent the μmoles of creatine per g of liver which had been replaced in the time interval between injection of the radio glycine and killing of the animal. Similarly, to estimate turnover rate of muscle creatine, the specific activity of muscle creatine was divided by the specific activity of liver creatine from the same animal and the result multiplied by the concentration of muscle creatine.

Fig. 1 presents the results of calculation of the turnover rate of liver creatine. It is apparent that in the E-deficient animals all the liver creatine had been renewed within 1.5 hours. In contrast, normal animals required approximately 4 hours to renew all of their creatine. These results show clearly that the rate of creatine synthesis is elevated in Vit. E-deficient rabbits. Such a conclusion has been reached by other workers(6).



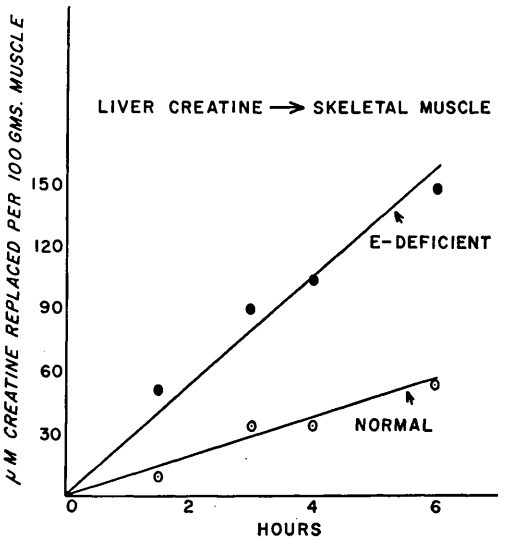


FIG. 1 (left). Creatine turnover in livers of normal and vit. E-deficient rabbits.
FIG. 2 (right). Creatine turnover in skeletal muscle of normal and vit. E-deficient rabbits.

The turnover rate of creatine in skeletal muscle is illustrated in Fig. 2. It is quite apparent that in Vit. E-deficient rabbits the turnover rate of muscle creatine is increased. This conclusion is in agreement with our earlier observation(1,2).

The turnover rate of heart creatine was also calculated and it was found to be unaffected by Vit. E deficiency. It should be mentioned however that heart creatine was found to be renewed at a considerably greater rate than the creatine of skeletal muscle.

The results of these experiments indicate that the creatinuria of Vitamin E deficiency is due, at least in large part, to an inability of the skeletal muscle to retain creatine after its incorporation. In preliminary experiments, it has been found that when normal and Vit. E-deficient rabbits are injected with inorganic P^{32} there is no depression of the incorporation of the P^{32} into phosphocreatine in the deficient animals. This strongly suggests that the inability of the dystrophic muscle to retain creatine is not due to an impaired phosphorylation. The explanation for this defect will probably require more basic information on the state of creatine in muscle and on the factors which influence its stability.

Summary. 1) Normal and Vit. E-deficient rabbits were killed at varying time intervals after injection of glycine-1-C^{14}. Concentration and specific activities of kidney glycocyamine, liver creatine, skeletal muscle creatine and heart creatine were determined. Vit. E deficiency led to an elevated concentration of liver creatine, to reduced concentration of skeletal muscle creatine and did not influence the concentration of kidney glycocyamine or heart creatine. 2. The turnover rate of liver creatine and skeletal muscle creatine was increased in Vit. E-deficient rabbits. The turnover rate of heart creatine was considerably greater than the turnover rate of skeletal muscle creatine and was unaffected by Vit. E deficiency. The results show that in Vit. E deficient rabbits, rate of creatine synthesis is increased and turnover rate of skeletal muscle creatine is increased.

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