Carnosinase Activity in Tissues of Normal and Dystrophic Rabbits.* (29171)

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It has been shown that vit. E-deficient diet (1,2) and also intramuscular injection of the ethyl ester of gamma-butyrobetaine (gBBE) (3) caused rabbits to excrete 1-methylhistidine in large amounts. Hosein(3) has suggested that gBBE either inhibits formation of anserine or increases its rate of hydrolysis. McManus(1) has shown a diminished concentration of muscle carnosine and anserine suggesting an abnormality in the metabolism of these dipeptides. She believes that vit. E-deficiency results in a hydrolysis of muscle anserine to beta-alanine and 1-methylhistidine as well as a decreased synthesis of anserine.

Absence of carnosinase activity from dystrophic rabbit muscle(1) and normal rat muscle(4) has been reported. It is possible, however, that carnosine and anserine are released from dystrophic muscle, translocated to other tissues, and there hydrolyzed by carnosinase, thereby contributing to the 1-methylhistidinuria. The studies reported here describe the carnosinase activities in tissues, *viz.*, spleen, kidney, liver, muscle, and heart of normal, vit. E-deficient, and gBBE injected rabbits and also the effects of gBBE on carnosinase *in vitro*.

Methods. Twenty-four rabbits were divided into 3 groups and were fed the following diets *ad lib:* A. Dystrophy-producing diet of Young and Dinning(5) as modified by Diehl(6) plus 8 mg alpha-tocopherol acetate thrice weekly, B. Dystrophy-producing diet, and C. Dystrophy-producing diet plus alpha-tocopherol plus 1 mg of gBBE dissolved in 1 ml of normal saline given daily by intramuscular injection. gBBE was prepared synthetically by the method of Hosein (3).

After symptoms appeared(7) animals were sacrificed by cervical dislocation and tissues of heart, kidney, liver, skeletal muscle, and spleen were quickly excised and weighed. The assay was that of Smith(8) including a preincubation with Mn^{++}. After 30 minutes incubation, aliquots were removed and titrated for freed carboxyl groups by the alcoholic microtitration method of Grassman and Heyde(9).

Aliquots of urine from gBBE-treated animals were assayed for 1-methylhistidine by the chromatographic system employed by Fink *et al*(2). To obtain the effect, if any, of gBBE *in vitro,* the substance was incorporated into the carnosinase assay system to concentrations as high as 1 M.

Results. Carnosinase activities in various tissues of normal and dystrophic rabbits are shown in Table I. Values are expressed as the zero-order constant, K_o, as per cent hydrolysis per minute and represent average of tissue from 8 animals on Diet A, 9 rabbits on Diet B, and 6 animals on Diet C. There were no significant differences in tissue carnosinase of animals receiving normal diet, vit. E-deficient, or those made dystrophic by gBBE injection ($p > .10$). The relative activity of carnosinase in tissues from the 3 different sets of animals were liver > kidney > spleen > muscle. The activity of muscle was so small as not to be significant and was observed in only about one-third of the tissues assayed. In no instance was any activity observed by heart homogenate.

Rabbits injected with gBBE showed 1-methylhistidinuria as early as the fifth day. Its identity was confirmed by comparison with chromatographed authentic material and these results would confirm those reported by Hosein(3).

The effect of gBBE upon carnosinase activity of tissue homogenates *in vitro* was shown to be purely ionic in character; at gBBE concentrations of greater than 0.1 M in the assay system, the activity fell off

* This investigation was supported by research grant, Am. Cancer Soc., So. Dakota Division.

TABLE I. Effect of Vitamin E-Deficiency and gBBE on Carnosinase Values of Rabbit Tissue. Values expressed as the zero-order constant, Ko, as per cent hydrolysis per minute and probable error of mean.

Tissue	Diet A Vit E-defic + vit E	Diet B Vit E-defic	Diet C Vit E-defic + vit E + gBBE
Spleen	.61 ± .07	.54 ± .03	.36 ± .12
Kidney	.87 ± .04	.81 ± .06	.74 ± .07
Liver	1.08 ± .05	1.09 ± .05	1.07 ± .03
Muscle	.07 ± .03	.06 ± .02	.025 ± .01
Heart	.0 ± .0	.0 ± .0	.0 ± .0

linearly to zero at gBBE of 1 M. This effect could be prevented by doubling of the concentration from 0.2 M to 0.4 M, of the pH 8.0 Tris buffer, in which normal carnosinase activity was demonstrable to gBBE concentrations of 1 M.

Discussion. These results show that, according to the assay used, there is no increase of carnosinase activity in tissues during conditions of muscle wasting. Thus, it would suggest that the 1-methylhistidinuria observed was not a result of increased hydrolysis of anserine by increased carnosinase activity.

It appears that the carnosine present in muscle is separated from the enzyme which in other tissues (spleen, liver, kidney) hydrolyzes it. For this reason it enjoys a protection unique among the proteinaceous constituents of muscle. By contrast with the lack of carnosinase in either normal or dystrophic muscle, there has been noted an increased activity of catheptic proteolytic enzymes(10), dipeptidases in dystrophic muscle(11), and more recently, increased activity of the lysosomal hydrolytic enzymes, including cathepsins, of muscles of genetically dystrophic mice and chickens(12) and vit. E-deficient rabbits (13). Perhaps in dystrophy the cathepsins that are released by lysosome lysis assume in muscle the function normally fulfilled by carnosinase elsewhere. Carnosinase could still be produced at a normal rate, even though its substrate, already being hydrolyzed by muscle cathepsins, no longer reaches the spleen, liver, or kidney. Further, the degree of saturation of the enzyme *in vivo* is unknown; if the enzyme is normally unsaturated, a slight increase in substrate concentration could easily be hydrolyzed without an increase in enzyme quantity.

Summary. 1. Carnosinase activity was measured in kidney, liver, spleen, heart and skeletal muscle of normal, vit. E-deficient, and gBBE treated rabbits. 2. No significant differences were noted in carnosinase values in tissues of animals on the 3 different regimens. 3. Rabbits injected with gBBE showed 1-methylhistidinuria. 4. gBBE had no effect upon carnosinase activities *in vitro*.

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Received February 2, 1964. P.S.E.B.M., 1964, v116.