# Effect of L-Histidine on Creatine, Histidine, and 1-Methylhistidine Excretion of Normal and Vit. E-Deficient Rabbits.* (26799)

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It has been shown(1,2) that Vit. E-deficient diet caused rabbits to excrete 1-methylhistidine in large amounts. Appearance of this amino acid in urine generally precedes that of creatine making it one of the earliest detectable symptoms of nutritional muscular dystrophy. 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.

Regardless of the causative mechanism, the loss of large amounts of 1-methylhistidine might itself produce secondary effects such as a significant drain on the body's reserve of the essential amino acid histidine. Our studies describe the effect of dietary L-histidine on excretion of creatine, histidine, and 1-methylhistidine of normal and Vit. E-deficient rabbits.

*Methods.* Twelve male rabbits, divided into 4 equal groups, were fed the following diets *ad lib.*: A. Dystrophy-producing diet of Young and Dinning(3) plus 8 mg alpha-tocopherol acetate thrice weekly: B. Dystrophy-producing diet containing 1% L-histidine plus 8 mg oral alpha-tocopherol acetate thrice weekly: C. Dystrophy-producing diet: D. Dystrophy-producing diet containing 1% L-histidine. When histidine was incorporated into the diet, an equivalent weight of sucrose was deducted.

Twenty-four hour urine samples were collected occasionally in a rabbit metabolism cage. Creatine and creatinine were determined by the method of Folin(4) while quantitative determinations of histidine and 1-methylhistidine were made by Dowex-50 chromatography using the method of Tallan *et al.*(5).

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*Results.* Urinary histidine and 1-methylhistidine values and creatine/creatinine ratios are shown in Table I. Values are expressed as $\mu$M/24 hr for the 2 amino acids. Rabbits of Group A excreted less histidine than those of Group B although the difference was not significant. No animals of Group A excreted any detectable amounts of 1-methylhistidine. Rabbit 6 of Group B did excrete this amino acid after 31 days on the diet. This same animal exhibited a creatine/creatinine ratio of 0.83 after 41 days on the diet while the remaining rabbits of both groups had insignificant ratios throughout the experiment.

Animals of Group C excreted significantly smaller amounts ($p<0.01$) of histidine than those animals on Diet A. One rabbit of Group C excreted none while the other 2 excreted less than half that of animals on Diet A. In contrast, those on Diet D excreted similar amounts to those on Diet B until about 3 weeks when histidine was no longer detectable. Animals on Diet C excreted large amounts of 1-methylhistidine within 2 weeks after being placed on the deficient diet. The amounts generally increased until death occurred. Rabbits fed Diet D excreted smaller amounts ($p>0.01<0.05$) of 1-methylhistidine than those on Diet C, *viz.*, after 3 weeks, an average excretion of 59 $\mu$M *vs* 165 $\mu$M. Animals on Diet C also excreted less histidine ($p>0.01<0.05$) than those on Diet D as well as exhibiting creatinuria within 2 weeks after being placed on the deficient diet, in contrast to Group D. Just before death, the creatine/creatinine ratios of those on Diet C rose to 4.7, 6.4, and 2.2 respectively, while animals on Diet D, at the same dates, showed ratios of 1.1, 0.2, and 1.5.

*Discussion.* Perhaps the most important point to be made from these experiments is that animals receiving an E-deficient diet containing 1% histidine excreted less urinary 1-methylhistidine than those not receiv-

TABLE I. Effect of Dietary L-histidine on Urinary Histidine and 1-methylhistidine, and Creatine/Creatinine Ratios of Normal and Vit. E-Deficient Rabbits. Values expressed as $\mu$M/24 hr.

| Diet | | Rabbit No. | Days on diet | Histidine | 1-methyl-histidine | Creatine / Creatinine |
|---|---|---|---|---|---|---|
| A | Vit. E-deficient + vit. E | 1 | 17 | 3.9 | .0 | .10 |
| | | 1 | 35 | 7.5 | .0 | — |
| | | 2 | 15 | 6.6 | .0 | .04 |
| | | 2 | 32 | 17.6 | .0 | .03 |
| | | 3 | 46 | 2.9 | .0 | — |
| B | *Idem* + 1% L-histidine | 4 | 12 | 14.0 | .0 | — |
| | | 4 | 32 | 13.4 | .0 | .36 |
| | | 5 | 10 | 8.0 | .0 | .08 |
| | | 5 | 13 | 19.2 | .0 | .10 |
| | | 6 | 31 | 10.0 | 3.3 | .35 |
| | | 6 | 41 | 10.7 | 24.2 | .83 |
| C | Vit. E-deficient | 7 | 16 | .0 | 64.2 | 1.44 |
| | | 7 | 19 | 2.6 | 73.5 | 4.71 |
| | | 8 | 12 | .0 | 176.8 | .37 |
| | | 8 | 22 | .0 | 292.7 | 1.45 |
| | | 8 | 24 | .0 | 406.6 | 6.42 |
| | | 9 | 13 | 2.0 | 222.6 | .98 |
| | | 9 | 22 | 1.9 | 128.0 | 2.19 |
| D | *Idem* + 1% L-histidine | 10 | 12 | 16.6 | 18.0 | — |
| | | 10 | 20 | .0 | 38.7 | .28 |
| | | 10 | 25 | .0 | 120.9 | 1.14 |
| | | 10 | 31 | .0 | 231.6 | .80 |
| | | 11 | 9 | 8.4 | .0 | — |
| | | 11 | 24 | .0 | 57.4 | .18 |
| | | 11 | 26 | 13.3 | 118.7 | .22 |
| | | 11 | 29 | 45.4 | 75.0 | 1.54 |
| | | 12 | 9 | 9.0 | .0 | — |
| | | 12 | 16 | 31.4 | 35.3 | .13 |
| | | 12 | 23 | .0 | 79.7 | 1.53 |

ing the supplement. Furthermore, the additive markedly reduced creatinuria and prolonged longevity an average of 16 days, or about 75% as compared to those on the deficient diet alone.

Also, animals fed normal diet did not excrete methylhistidine. This would agree with some findings(2,6); however not with other workers(1,5,7) who found a low content of 1-methylhistidine in normal rabbit urine. It should be stressed that rabbit 6 did excrete this derivative while receiving alpha-tocopherol, but also had some creatinuria. This would infer a mild dystrophy, although at no time were physical symptoms obvious.

It might be expected that rabbits on Diet B would excrete more histidine than those not receiving the supplement on Diet A since both sets consumed similar amounts of diet with the former thus receiving about 0.4 g per day more histidine than did the latter group. Results further indicate that animals on an E-deficient diet excreted little histidine in the urine but rather massive amounts of 1-methylhistidine. The latter finding would agree quantitatively with those of Fink *et al.* (2) that dystrophied rabbits excreted as much as 400 $\mu$M/24 hr/kg body weight of the methyl derivative. Their results were about 5-fold those reported elsewhere(1).

McManus(1) has shown that normal and dystrophied muscle are devoid of carnosinase activity, and suggested that anserine could be hydrolyzed elsewhere to cause methylhistidinuria in E-deficient animals. This would deplete histidine from the various metabolic pools and could explain Tallan's(8) findings of a decrease in free histidine of muscle of severely E-deficient rabbits. The possibility also exists that there is an accelerated rate of protein breakdown to furnish needed histidine. This would account for the significant rise in free amino acids of various tissues as shown by Smith and Nelson(9).

It is conceivable that supplementation of the deficient diet with histidine would retard the dystrophic process by maintaining a high level of histidine in the metabolic pool. This would result in a diminished creatinuria and methylhistidinuria.

*Summary.* 1. Effects of L-histidine on excretion of 1-methylhistidine, histidine, and creatine of normal and Vit. E-deficient rabbits have been studied. 2. L-histidine caused an increased excretion of histidine from animals on a normal and Vit. E-deficient diet. 3. Three rabbits on a normal diet and 2 of 3 on a normal diet + L-histidine failed to excrete 1-methylhistidine in the urine. 4. Vit. E-deficient animals excreted small amounts of histidine, but large quantities of 1-methylhistidine and creatine. 5. Vit. E-deficient rabbits fed 1% L-histidine excreted less 1-methylhistidine and creatine, but increased amounts of histidine. 6. 1% L-histidine was shown to prolong the lives of Vit. E-deficient rabbits.

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## Studies on Platelets XXIV. Analysis of Some Platelet Antigens by Complement Fixation Test.* (26800)

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Platelets exhibit species specificity(1) and very likely cell(2) and blood group(3,4) specificity. Specific antigens proper to platelets only may also be found(5,6). Evidence for organ specificity of platelets has also been presented. Thus, injection of serum from patients with chronic idiopathic thrombocytopenic purpura may induce thrombocytopenia in dogs(7) and rabbits(8), an effect which is abolished if the serum is previously absorbed with human platelets. Also, the platelet agglutinins found in human idiopathic thrombocytopenic purpura are absorbed by monkey platelets(9), while antiguinea pig platelet rabbit serum agglutinates human platelets as well(10). Finally, there is a common antigenic structure of platelets obtained from man, goat, sheep, horse and pig(11). Most of the previous work *in vitro,* however, has been carried out using agglutination procedures. This report presents an analysis of the organ specificity of platelets using a more reliable technic, namely complement fixation.

*Materials and methods.* (a) *Preparation of platelets.* Blood was collected from the antecubital vein (man) or jugular vein (horse, cow and dog) into plastic bags with ACD solution or into silicone-coated bottles containing 0.1 volume of 1% Sequestrene-$Na_2$ solution. Platelets were separated and washed 5× with isotonic saline by standard technic(6). Large amounts were frozen at −4°C until used for immunization purposes.

(b) *Preparation of platelet antigen for complement fixation test.* A platelet button

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