15062

# A Further Study of the Lability of the Methyl Group of Creatine.

SOFIA SIMMONDS AND VINCENT DU VIGNEAUD.*

*From the Department of Biochemistry, Cornell University Medical College, New York City.*

It has been shown by du Vigneaud, Chandler, and Moyer[1] that neither creatine nor creatinine will support the growth of young rats maintained on an otherwise methyl-free diet in which homocystine is the only sulfur-amino acid. The inability of rats to grow under these conditions was indicative of the non-reversibility of transmethylation from methionine[2,3] and choline[3] to creatine. However, it was recognized[1] that the transfer of methyl groups from creatine to choline and methionine might occur, but not at a rate fast enough to support growth. To test this possibility, creatine containing the deuteriomethyl group was synthesized and fed to young rats to see whether it could act as a methyl donor for choline synthesis.

*Synthesis of deuteriocreatine.* Deuteriosarcosine hydrochloride was synthesized by the method of Fischer and Bergmann[4] using deuteriomethyl iodide[3] for the methylation of toluenesulfonyl glycine. Deuteriosarcosine then was prepared from the hydrochloride by treatment with $Ag_2O$, and was converted to deuteriocreatine by treatment with cyanamide in the presence of a trace of ammonia.[5] The deuteriocreatine contained 81.0 atom % deuterium in the methyl group.

*Analysis.* Deuteriocreatine. Calculated, N 31.46; found, N 31.53.

*Feeding experiment.* Two albino rats weighing about 50 g were placed in individual cages arranged for urine collection and were fed a basal diet containing homocystine but lacking methionine, choline, and betaine. The basal diet was the same as that employed in the previous growth studies with ordinary creatine.[1] Deuteriocreatine was mixed in the diet at a level of 1.75 parts per hundred. This provided a daily supplement of creatine equivalent in methyl groups to approximately 25 mg of choline chloride. For the feeding experiment, the synthetic deuteriocreatine was diluted with a sufficient amount of ordinary creatine in order to lower the deuterium content of the methyl group from 81.0 to 64.3 atom %.

The changes in body weight and amounts of test compound ingested during the feeding period are given in Table I. The rats were sacrificed at the end of 7 days and choline and creatine were isolated from the tissues of each by the procedures described in a previous investigation.[3] Choline was isolated as choline chloroplatinate, and creatine as creatinine potassium picrate. The purity of the latter compound was determined colorimetrically by the Jaffe reaction.

*Analysis.* Choline chloroplatinate.

Rat No. 1012 Calculated, Pt. 31.66; found, Pt. 31.41.

Rat No. 1013 Calculated, Pt. 31.66; found, Pt. 31.63.

The urine excreted by both rats over the entire experimental period was collected and pooled, and creatinine was isolated from it as creatinine potassium picrate.[6] The deuterium content of each of the isolated compounds is given in Table I.

*Discussion.* As the data in Table I show, the tissue choline contained no deuterium. The methyl group of creatine, therefore, is not available for the synthesis of choline, and transmethylation from choline to creatine is

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1 du Vigneaud, V., Chandler, J. P., and Moyer, A. W., *J. Biol. Chem.*, 1941, **139**, 917.

2 Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, 1940, **132**, 559.

3 du Vigneaud, V., Cohn, M., Chandler, J. P., Schenck, J. R., and Simmonds, S., *J. Biol. Chem.*, 1941, **140**, 625.

4 Fischer, E., and Bergmann, M., *Ann. Chem.*, 1913, **398**, 96.

5 Rosengarten, F., and Strecher, A., *Ann. Chem.*, 1871, **157**, 1.

TABLE I.
Feeding of Deuteriocreatine.
Deuteriocreatine containing 64.3 atom % deuterium in the methyl group was fed for 7 days.

| Rat No. | Change in body wt, g | Total deuteriocreatine ingested, mg | Deuterium content of isolated compounds: Choline chloroplatinate from tissues, atom % | Creatinine K picrate from tissues, atom % | Creatinine K picrate from urine, atom % |
|---|---|---|---|---|---|
| 1012 | 55-58 | 542 | 0 | 7.3 ±.1 | 11.5 ±.2 |
| 1013 | 50-54 | 438 | 0 | 6.7 ±.1 | |

thus irreversible.

The creatinine potassium picrate isolated from the rat tissues at the end of the experimental period contained about 7 atom % deuterium. The creatinine potassium picrate isolated from the pooled urine of the entire experimental period contained even more deuterium, *i.e.*, 11.5 atom %. These data on urinary creatinine agree with the findings of Bloch and Schoenheimer.[6] These investigators found that although urinary creatinine from rats on a *creatine-free* diet is derived solely from tissue creatine, the urinary creatinine excreted during the feeding of relatively large amounts of creatine labeled with $N^{15}$ contained more than the expected amount of $N^{15}$. Bloch and Schoenheimer, therefore, suggested that a small portion of dietary creatine is converted to creatinine and excreted before the dietary creatine is completely admixed with the creatine in the tissues. Our results offer support for this suggestion; for, in the absence of the direct conversion and excretion of a fraction of the deuteriocreatine of the diet, the deuterium content of the urinary creatinine should have been 3.5 to 4 atom %, rather than the 11.5 atom % actually found.

It is of interest to point out that on the basis of the amount of deuterium in the tissue creatine, an average of 43% of the tissue creatine was derived from the deuteriocreatine of the diet in 7 days.

*Summary.* Creatine containing the deuteriomethyl group was fed to two young rats on an otherwise methyl-free diet. The creatine isolated from the tissues contained a considerable amount of deuterium, while the choline from the tissues was completely devoid of deuterium. Transmethylation from choline to creatine, therefore, is irreversible.

[6] Bloch, K., and Schoenheimer, R., *J. Biol. Chem.*, 1939, **131**, 111.