THE EFFECT OF METHYLTESTOSTERONE ON THE RATE OF SYNTHESIS OF CREATINE*

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After the daily administration of methyltestosterone to normal human subjects for several weeks, creatine usually appears in the urine in gradually increasing amounts (1, 2). Because the creatinuria so induced is associated with an increase in the concentration of serum creatine, and also because the amount of urinary creatinine increases, it has been generally assumed that the synthesis of creatine is accelerated by methyltestosterone.

The effect of methyltestosterone on the metabolism of creatine can be more directly observed by measuring the rate of turnover of creatine by isotope methods. It has previously been shown that the rate of dilution of labeled body creatine, as reflected in the concentration of isotope in urinary creatinine, is a measure of the rate of replacement of depot creatine by endogenous creatine (3–5). By so tagging the body creatine of a normal male subject subsisting on a diet as free of creatine and creatinine as possible, the rate of synthesis of creatine before and during the administration of methyltestosterone has been measured. Evidence will be presented that methyltestosterone does increase the rate of synthesis of creatine. That this is specifically brought about by its effect on the reaction or reactions involved in the formation of guanidoacetic acid is indicated by other data.

EXPERIMENTAL

2 weeks before the administration of isotopic creatine the subject (H. D. H.) was placed on a diet free of meat or meat products. Milk consumption was limited to 250 ml. per day. The creatine of the body was then labeled with isotopic nitrogen by the intravenous administration of 1.67 gm. of creatine (19.4 mg. per kilo) containing 31 atom per cent excess N\textsuperscript{15} and 1.5 gm. of guanidoacetic acid (17.5 mg. per kilo) containing 30.5 atom per cent excess N\textsuperscript{15}, both in the α position. The isotopic creatine was synthesized as described (5, 6); the N\textsuperscript{15} guanidoacetic acid was pre-

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pared by the method of Brand and Brand (7). Isolation of urinary creatinine for the analysis of $N^{15}$ was begun on the 5th day after the isotopic creatine was given, and from that time every 7th day for 49 days. During the 6 intervening days of each period, urine specimens were pooled and analyzed for creatine, creatinine, and guanidoacetic acid. The method of Peters (8) was used for the measurement of creatine and creatinine, and that of Hoberman (9) for guanidoacetic acid. The isolated creatinine was degraded to sarcosine, as described (5). The sarcosine, isolated as the toluene sulfonyl derivative, was analyzed for $N^{15}$ in a mass spectrometer constructed according to the specifications of Nier (10).

![Graph showing changes in the isotope concentration of urinary creatinine observed before and during the administration of methyltestosterone. The broken line is an extrapolation of the curve obtained during the control period.](http://www.jbc.org/)

A control period of 20 days followed the day on which the first sample of creatinine was isolated. Thereafter 50 mg. of methyltestosterone were taken daily by mouth for 28 consecutive days.

**Results**

The changes in the isotope concentration of the urinary creatinine during the control period of 21 days and during the ensuing 28 days on methyltestosterone are shown in Fig. 1. The slope of the straight line from $t = 0$ to $t = 21$, obtained by plotting the data semilogarithmically, is 0.0143. This has been shown to be the fraction of the total body creatine which undergoes replacement each day (5). Since the average creatinine excretion during the control period was 1.76 gm. per day, equivalent to 2.04 gm. of creatine per day, the average total body creatine during this period is calculated to be $2.04/0.0143 = 143$ gm.
After methyltestosterone has been taken for 1 week, the slope of the curve of Fig. 1 becomes steeper, denoting a more rapid rate of dilution of body creatine than during the control period. In a previous paper (5) it was shown that, for the condition of a changing total body creatine, labeled with isotope, the amount of creatine at any time \( t \) can be calculated by the substitution into the equation

\[
G = \frac{C_0 G_0}{C} e^{-kt}
\]

where \( C_0 \) and \( C \) are the isotope concentrations of the urinary creatinine at time \( t = 0 \) and \( t \), respectively; \( G_0 \) and \( G \) are the amounts of total body creatine at \( t = 0 \) and \( t \), respectively, and \( k \) is the turnover constant. For the application of this equation to the present study it has been assumed that methyltestosterone does not alter the fraction of the total body creatine which undergoes conversion to creatinine. With \( C_0 = 0.400 \) atom per

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>( ^{15}N ) in creatinine</th>
<th>Total body creatine (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.400</td>
<td>143</td>
</tr>
<tr>
<td>7</td>
<td>0.345</td>
<td>150</td>
</tr>
<tr>
<td>14</td>
<td>0.307</td>
<td>152</td>
</tr>
<tr>
<td>21</td>
<td>0.288</td>
<td>158</td>
</tr>
<tr>
<td>28</td>
<td>0.230</td>
<td>167</td>
</tr>
</tbody>
</table>

TABLE I

Changes in Body Creatine Observed during Ingestion of Methyltestosterone

It is apparent that the body depots gained 24 gm. of creatine in the period during which methyltestosterone was taken. The average rate of synthesis of creatine therefore increased approximately 40 per cent.

Graphical integration of the data of the last column in Table I permits calculation of the average expected creatinine excretion according to

\[
\bar{d} = \bar{k} \bar{G}
\]

where \( \bar{d} \) is the average expected creatinine excretion (expressed as gm. of creatine per day), and \( \bar{G} \) is the average number of gm. of creatine in the body, the integration being performed over the 28 day period during which methyltestosterone was given. By this method \( \bar{d} \) was calculated to be
2.19 gm. per day. The average observed creatinine excretion during the same time interval was 1.85 gm. per day, equivalent to 2.14 gm. of creatine per day. The agreement between the calculated and observed creatinine excretion supports the assumption that during the period under observation methyltestosterone was without effect on the process concerned with the transformation of creatine to creatinine.

Although creatine could not be detected in the urine at any time during the experimental period, the urinary excretion of guanidoacetic acid increased approximately 70 per cent while methyltestosterone was administered. This is shown in Fig. 2. The rise in the excretion of urinary guanidoacetic acid which occurred during the control period is not significant, since variations of this order of magnitude have not been uncommon in our experience. However, normal male urine has never been found to contain such large amounts of guanidoacetic acid as were excreted during the administration of methyltestosterone.

**DISCUSSION**

It is significant that the rate of creatine synthesis increased during the 1st week of methyltestosterone administration. The fact that several weeks usually elapse before methyltestosterone induces creatinuria is believed to be related to the amount of creatine already present in the body tissues. Samuels et al. (2) found it possible to shorten this latent period by incorporating extra creatine in the diet. The absence of a detectable amount
of creatine in the urine after 4 weeks of ingestion of methyltestosterone can be attributed to the omission of creatine from the diet.

A rise in the amount of guanidoacetic acid excreted in the urine of normal children has been reported by Hoagland et al. (11) to follow the administration of methyltestosterone. The excretion of greater amounts of guanidoacetic acid during the period of administration of methyltestosterone than during the control period indicates that the step which determines the rate of synthesis of creatine, at least under the conditions brought about by the ingestion of methyltestosterone, is related to the methylation of guanidoacetic acid. Borsook et al. (12) have presented evidence that guanidoacetic acid is formed in the kidneys in the human. Methyltestosterone administered to patients with severe nephritis induced neither creatinuria nor a significant rise in serum creatine (2). These observations are consistent with the hypothesis that methyltestosterone increases the rate of formation of creatine specifically by its effect on the synthesis of guanidoacetic acid. There is therefore no necessity of postulating that methyltestosterone acts in any way as a "methylating catalyst" (13).

That the rate of creatine turnover is independent of the methionine content of the diet has been observed by Cohn et al. (4). It would appear that the rate of synthesis of creatine is determined under their conditions either by the rate of synthesis of guanidoacetic acid or by the concentration of the enzyme or enzymes available for the process of methylation. Further it has been observed in the human that oral tolerance to guanidoacetic acid is not increased by the administration of an equivalent amount of methionine.¹ It would therefore seem most probable that during the administration of methyltestosterone the rate of synthesis of creatine is limited by the concentration of catalysts required for the methylation of guanidoacetic acid.

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SUMMARY

The effect of methyltestosterone on the rate of synthesis of creatine has been studied in a normal human subject by means of isotope methods. Evidence is presented that methyltestosterone promptly brought about an increase in the rate of synthesis of creatine. That this is specifically due to the effect of methyltestosterone on the reaction or reactions involved in the synthesis of guanidoacetic acid is indicated by other data.

¹ Unpublished observations.
BIBLIOGRAPHY

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