STUDIES ON THE METABOLISM OF CREATINE AND CREATININE

III. FORMATION OF CREATINE BY ISOLATED RAT TISSUES*

BY ZELMA BAKER and BENJAMIN F. MILLER

(From the Department of Medicine of the University of Chicago, Chicago)

(Received for publication, November 3, 1939)

The precursors, mechanism, and site of creatine formation are still uncertain, even though they have received the attention of investigators for many years. An examination of the results on creatine formation summarized in Hunter's monograph (1928) shows the many contradictions in this field. In the 10 years which have elapsed since the publication of this work, few experiments in vivo have been performed which clarify the problem. On the other hand, several workers have reported recently the synthesis of creatine by tissues in vitro. The observations of Borsook and Jeffreys (1935) indicate that creatine is formed by liver, kidney, diaphragm, and intestine, and that the rate of formation can be increased in liver by the addition of a complete hydrolysate of egg albumin. Fisher and Wilhelmi (1937) observed an increase in creatine after perfusing the isolated rabbit heart with arginine. Bach (1939) reported an apparent increase in creatine after incubation of chopped rat heart with glycocyamine and glycine.

The specificity of the methods employed in the above investigations is open to some question. We present in this communication results on creatine formation by rat tissues in vitro, obtained by employing the specific, enzymatic method of Miller, Allinson, and Baker (1939) for the estimation of creatine. The capacity of various tissues to form creatine, without addition of possible precursors, has been observed.

EXPERIMENTAL

The rats used in these experiments were normal, adult males. For purposes of comparison, a few experiments were performed

* Aided by a grant from the John and Mary R. Markle Foundation.
Creatine and Creatinine. III

with adult females. The animals were killed by decapitation, and the tissues were rapidly excised. In most of the experiments the tissues were cut into small fragments on a cold plate after removal of adherent fat and blood. For experiments with sliced tissue, slices of uniform thickness (0.3 to 0.4 mm.) were prepared by free-hand section, and stored in a moist chamber until ready for use. Excess moisture was removed from the slices with filter papers before weighing. The experiments were performed with no added substrate in Ringer-phosphate or Ringer-bicarbonate medium, prepared according to Krebs (1931). Suitable amounts of tissue were rapidly weighed and immersed in 5 cc. of medium, contained in 50 cc. Erlenmeyer flasks. The contents of the flasks were saturated with oxygen or oxygen-carbon dioxide mixture (or nitrogen-carbon dioxide for anaerobic experiments). The flasks were stoppered and shaken in a water bath at 38° for 2 to 4 hours. The pH of the medium remained relatively constant throughout the entire incubation period. Depending on the amount of tissue used, either 10 or 20 cc. of 2 N sulfuric acid were pipetted into the flasks, and the estimation of creatine was carried out as described by Miller, Allinson, and Baker. All experiments were set up in duplicate, and the analysis of each sample was performed in duplicate. Samples of tissue were killed with sulfuric acid at the start of the experiment to give the initial creatine concentration. The value obtained after incubation represents the final creatine concentration. The difference between these two values is the amount of creatine synthesized by the tissue. Since, in this method, the tissue cannot be removed for drying at the end of the experimental period, all results are expressed in terms of original wet weight of tissue.

Results

Typical results obtained with various rat tissues in the absence of added substrate are given in Tables I and II. All values represent the average of determinations on duplicate samples of tissue. The data show that only liver and kidney produce significant amounts of creatine from precursors normally present in the tissue. The percentage increases in kidney and liver are very striking, averaging approximately 10 to 20 per cent for the former and 10 to 35 per cent for the latter tissue. No significant percentage in-
creases were observed in brain, testis, spleen, and muscle (cardiac, skeletal, and intestinal muscles were studied). As can be seen from Table I, very slight absolute increases were occasionally observed in muscle creatine. However, the high initial creatine content of this tissue interferes with the precise estimation of small increases of creatine. Nevertheless, even though it is question-

**Table I**

Formation of Creatine by Rat Muscle, Brain, Testis, and Spleen

<table>
<thead>
<tr>
<th>Tissue*</th>
<th>Buffer</th>
<th>Incubation period</th>
<th>Creatine, mg. per 100 gm. wet weight of tissue</th>
<th>Increase per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hrs.</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>Phosphate</td>
<td>2</td>
<td>202</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>221</td>
<td>217</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>198</td>
<td>198</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>Bicarbonate</td>
<td>4</td>
<td>174</td>
<td>174</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>407</td>
<td>410</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>409</td>
<td>415</td>
</tr>
<tr>
<td>Intestinal</td>
<td></td>
<td>4</td>
<td>322</td>
<td>327</td>
</tr>
<tr>
<td>muscle</td>
<td></td>
<td>4</td>
<td>101</td>
<td>100</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td>3</td>
<td>119</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>119</td>
<td>121</td>
</tr>
<tr>
<td>Testis</td>
<td>Phosphate</td>
<td>2</td>
<td>301</td>
<td>302</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>328</td>
<td>328</td>
</tr>
<tr>
<td>Spleen</td>
<td>Phosphate</td>
<td>3</td>
<td>19.8</td>
<td>19.9</td>
</tr>
<tr>
<td></td>
<td>Bicarbonate</td>
<td>6</td>
<td>19.8</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>18.1</td>
<td>18.0</td>
</tr>
</tbody>
</table>

* All experiments were performed with chopped tissue.

As shown in Table II, the amount of creatine synthesized per 100 gm. of wet weight of kidney tissue is the same for male and female rats. It is further shown that chopped and sliced kidney tissues are equally efficient in the production of creatine.

The following experiment suggests that an enzyme system is involved in the formation *in vitro* of creatine. Kidney tissue,
inactivated by heating at 80° for 10 minutes, produced no creatine upon subsequent incubation at 38° for 4 hours; unheated kidney tissue from the same animal produced 4.4 mg. per 100 gm. Further, the synthesis is completely inhibited by $10^{-3}$ M potassium cyanide,\textsuperscript{1} and partially inhibited by anaerobiosis.

That the increase is due almost entirely to creatine, and not to creatinine, was shown by an experiment on kidney tissue in which

\textsuperscript{1} Cyanide interferes with the Jaffé reaction. Therefore, it was completely removed by distillation before the determination of creatine.

\begin{table}
\centering
\caption{Formation of Creatine by Rat Liver and Kidney}
\begin{tabular}{|c|c|c|c|c|}
\hline
Tissue & Buffer & Incubation period & Creatine, mg. per 100 gm. wet weight of tissue & Increase & Remarks \\
\hline
\hline
Liver & Phosphate & 2 hrs. & 4.6 & 0.1 & +38 \\
& Bicarbonate & 3 & 7.1 & 8.0 & +13 \\
& & 4 & 5.5 & 6.6 & +20 \\
& & 4 & 9.9 & 13.3 & +34 \\
& & 2 & 34.6 & 38.4 & +11 \\
& & 3 & 52.0 & 55.6 & +7 \\
& & 6 & 52.0 & 58.9 & +13 \\
& & 4 & 29.3 & 34.6 & +18 \\
& & 3 & 38.7 & 46.4 & +15 \\
& & 4 & 26.3 & 30.3 & +15 \\
& & 4 & 24.0 & 28.5 & +19 \\

Kidney & Phosphate & 4 hrs. & 44.3 & 48.1 & +9 \\
& Bicarbonate & 45.2 & 54.3 & +16 \\
& & 4 & 36.1 & 42.8 & +19 \\
& & 3 & 34.3 & 40.8 & +19 \\
& & 4 & 31.3 & 36.8 & +18 \\
& & 31.3 & 33.9 & +8 \\
& & 4 & 55.1 & 59.4 & +8 \\
& & 53.5 & +8 \\
& & 10\textsuperscript{-3} M KCN. & & & \\
\hline
\end{tabular}
\end{table}
both creatine and creatinine were determined after a 4 hour incubation period. (Creatinine was determined by the method of Miller, Allinson, and Baker.) The increase in creatine plus creatinine was 7.7 mg. per 100 gm. Of this, 7.5 mg. were creatine, and only 0.2 mg. creatinine.

It is interesting to note that the absolute quantities of creatine produced are approximately 1 to 2 mg. per 100 gm. of kidney tissue per hour, and 0.3 to 0.8 mg. per 100 gm. for liver. Since it is likely that the process is considerably more efficient in vivo than in tissue slices and minces, it is possible that the kidney and liver play an important rôle in the production of creatine under normal physiological conditions.

SUMMARY

The formation of creatine by isolated rat tissues without added substrate has been studied. Kidney produced 4 to 8 mg. per 100 gm., and liver, 1.2 to 3.2 mg. per 100 gm. in a 4 hour incubation period. These values represent approximately 10 to 30 per cent increases. The process of creatine formation is apparently enzymatic in nature: it is heat-labile and is inhibited by cyanide and by anaerobiosis.

In other tissues studied, cardiac, skeletal, and intestinal muscle, brain, testis, and spleen, no significant percentage increases of creatine were found. (It is impossible with present methods to detect small, absolute increases in tissues of high creatine content such as cardiac and skeletal muscle.)

All analyses were performed with a specific, enzymatic method for the determination of creatine.

BIBLIOGRAPHY

STUDIES ON THE METABOLISM OF CREATINE AND CREATININE: III. FORMATION OF CREATINE BY ISOLATED RAT TISSUES
Zelma Baker and Benjamin F. Miller


Access the most updated version of this article at http://www.jbc.org/content/132/1/233.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/132/1/233.citation.full.html#ref-list-1