THE BIOLOGICAL PRECURSORS OF CREATINE*

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Most of the earlier investigations on the biological precursors of creatine and creatinine have employed balance experimentation on normal humans or animals. After administration of hypothetical precursors; the amounts of creatine (or creatinine) excreted were measured. None of these experiments has clearly revealed the precursors. The results were interpreted (3) as support of the view that creatine formation cannot be increased above the physiological needs of the cells by administration of amino acids. There are two other types of experiments, however, that had definitely pointed to glycine and arginine as creatine precursors. Brand et al. (4) found that patients with muscle dystrophy (in contrast to normal humans or animals) excrete more creatine after administration of glycine, and Fisher and Wilhelmi (5) claimed that the addition of arginine to the perfusion fluid of rabbit hearts resulted in creatine formation. We abstain in the introduction from presenting the vast literature on creatine formation, which has recently been completely reviewed (3, 6, 7). We shall discuss some of these papers and also the recent revealing experiments of Borsook and Dubnoff on arginine (8), in conjunction with our own experiments.

The only substance which is known to result in extra creatine (or creatinine) formation after administration to normal animals or humans is guanidoacetic acid ((6) p. 224). While this sub-

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Some of the results have been published in preliminary papers (1, 2).

1 The later experimental results and theories of Beard and collaborators, which would require opposite conclusions, are discussed in an addendum to this paper.
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stance is not a normal food constituent but has been found in small quantitics in normal urine and tissues (9, 10), it may be regarded as an intermediate in creatine formation. In the biological synthesis of creatine the following two steps thus appear to be established: (a) formation of guanidoacetic acid, and (b) methylation.

The formation of the total creatine molecule is a slow process; an amount corresponding to only about 2 per cent of the total creatine is synthesized per day by animals on a creatine-free diet (11). Reaction (b), methylation, however, proceeds rapidly, as a considerable part of administered guanidoacetic acid is immediately converted into creatine. The rate of creatine formation thus seems to be mainly restricted by the slow rate of reaction (a). Once guanidoacetic acid is formed, in the normal metabolism, it should be easily methylated.

Reaction (b), methylation, has recently been elucidated. Borsook and Dubnoff (12) have found that guanidoacetic acid was slowly converted into creatine by tissue slices, but that addition of methionine vastly increased the rate and extent of this process. None of the other compounds investigated had the effect of methionine. The findings were interpreted as indicating a shift of the methyl group from methionine to guanidoacetic acid to form creatine. The occurrence of this reaction in vivo has been established conclusively by du Vigneaud et al. (13). The administration of methionine containing deuterium in the methyl group resulted in the formation of deuto creatine (and creatinine).

The present paper is concerned with the source of nitrogen for creatine; i.e., with the precursors of guanidoacetic acid.

We have tested twelve nitrogenous compounds, most of which are normal body constituents, as creatine precursors by adding a small amount of their isotopic analogues to the ordinary stock diet of normal rats. We have thereby followed normal creatine formation. The synthesis of some of the isotopic compounds employed has been described before; the methods of preparation of the others are given in this paper. Most experiments were carried out with adult male rats which, as has been shown previously, synthesize an amount corresponding to about 2 per cent of the total body

2 The authors would like to express again their indebtedness to Dr. H. C. Urey for the valuable gift of isotopes, and especially for the high concentrations of N\textsuperscript{15} which were employed in the present study.
creatine per day. Only a few experiments were performed with immature, growing animals, which synthesized a larger fraction. The addition of the isotopic compound extended over several days, after which the animals were killed and creatine was isolated from the bodies. In a few experiments creatinine was isolated from the urine. The isotope content of the isolated compounds was determined. As the rate of creatine formation is slow, the amount of isotopic creatine formed from the respective precursors during the short experimental period could not have been large, even if all of the newly formed creatine had been derived exclusively from the isotopic dietary addition. The isotopic amino acids tested, before being utilized, must have mixed with considerable quantities of their normal, non-isotopic analogues present in the animal tissues or in the casein of the diet, and dilution of the isotope concentration in the nitrogenous source for creatine must thereby have occurred.

Of the normal dietary and tissue components tested, arginine and glycine were found to be exceptionally effective as creatine precursors. The nitrogen of both compounds is thus utilized for creatine formation. They provide nitrogen, however, to different parts of the creatine molecule. Creatine has 3 nitrogen atoms. The presence of the isotope marker in the total creatine molecule did not by itself indicate the mechanism of its formation unless the position of the isotope in the creatine was located. For this purpose some of the isotopic creatine samples were degraded by boiling with aqueous barium hydroxide as described before (11), whereby the nitrogen of the amidine group was recovered as ammonia and the remaining nitrogen atom as sarcosine (I).

\[
\begin{align*}
\text{NH}_2 & \\
\text{C–NH} & \xrightarrow{\text{Ba(OH)}_2} \text{Ba(OH)}_2 \\
\text{N–CH}_3 & \quad 2\text{NH}_3 + \text{CO}_2 + \\
\text{CH}_3\text{COOH} & \quad \text{HN–CH}_3
\end{align*}
\]

(1)

This becomes apparent from those series of experiments listed in Table I in which the same amount of isotopic compound, e.g. glycine or guanidoacetic acid, was given to adult and immature rats. The isotopic content in the creatine of the bodies of the latter was about 3 times as high, indicating that the immature animals had synthesized about 3 times as much creatine; i.e., about 6 per cent.
Separate isotope analysis of these two fractions revealed their origin. The amidine nitrogen of creatine is derived from that of the corresponding group of arginine, and the nitrogen of the sarcosine group originates from glycine.

**Compounds Tested**

**Guanidoacetic Acid**—The compound tested was prepared by the reaction of non-isotopic cyanamide with isotopic glycine (14). Following its addition in small amounts to the stock diet, both the creatine in the muscle and creatinine in the urine were found to have a high isotope content. The quantitative results of this and the following experiments (Table I) confirm the many reports that this substance is readily converted, i.e. methylated, to creatine. Its high effectiveness becomes apparent when the result is compared with an analogous experiment in which an equivalent amount of creatine (instead of guanidoacetic acid) was given (15). In both experiments the muscle creatine of the animals had about the same isotope concentration. Guanidoacetic acid when given in small amounts does not seem to be decomposed to an appreciable extent, as the urinary urea contained no marked nitrogen.

**Hydantoic Acid and Methylhydantoic Acid**—These two compounds were prepared by the action of normal potassium cyanate on isotopic glycine and sarcosine respectively (16, 17). Creatine isolated after their administration did not contain any marked nitrogen. The finding is in agreement with those of earlier investigators (18, 19), who failed to detect an increase of creatine formation or creatinine excretion after the feeding of these substances. In contrast to guanidoacetic acid, they cannot be considered as intermediates; their ureido group (NH$_2$C=O) is not converted into the guanido (NH$_2$C=NH) group in the body.

On the other hand, analogous reactions with other substances may be possible: Klose and Almquist (20) have recently demonstrated the direct conversion in the chick of citrulline into arginine.

**Ammonia**—When given in moderate amounts (21) to adult...
rats ammonia has a small effect as a source of creatine nitrogen. The isotope concentration in the compound isolated is just above the limit of error of the isotope analysis. However, by changing the experimental procedure creatine samples with a moderate isotope content can be obtained. This was done by employing immature rats and keeping them on a nitrogen-low basal diet.

The marked ammonia nitrogen represented 70 per cent of the total dietary nitrogen. Under these experimental conditions the utilization of other possible creatine precursors must have been relatively slight.

The isotopic creatine obtained from these animals was degraded. Most of the isotope was located in the amidine group, while the sarcosine contained only traces. The small amounts of nitrogen which had been utilized were thus mainly employed for the formation of the amidine group. It will be shown below that arginine had acted as an intermediate.

**dl-Tyrosine, l(-)-Leucine, and dl-Glutamic Acid—**Ammonia nitrogen has been shown to be utilized in a similar way, but to a lesser extent than amino acid nitrogen, for the reversible amination of amino acids (21). Amino acids which are not specific and direct precursors of creatine may thus be expected, like ammonia, to yield only a very small amount of their nitrogen for creatine formation. The creatine samples isolated after administration of the three amino acids had either no marked nitrogen or only traces. The compounds tested thus seem to be about as effective as ammonia. Creatine isolated after feeding l(-)-leucine was degraded, and most of the isotope was found to be present in the amidine group. The values, however, are so low that they include a considerable error.

**Glycine—**In contrast to ammonia and the three amino acids discussed, glycine is a highly effective creatine precursor. This is in agreement with the theory of Brand et al. (4). Its administration in small amounts results in the formation of creatine with a high isotope content. The isotope constitution of the sample is fundamentally different from that obtained after ammonia feeding. Most of the isotope is located in the sarcosine part and only traces in the amidine group. Glycine may thus be considered

4 The experiment, carried out mainly for other purposes, has been discussed earlier (22).
as a specific precursor of the "sarcosine part" of the creatine molecule.

Arginine—Like creatine, arginine contains a guanido group and has been claimed by many investigators to be a precursor of creatine. The results obtained with balance experiments, however, were contradictory and, as stated above, did not give clear cut results. Recently Fisher and Wilhelmi (5) reported the perfusion of rabbit hearts with arginine to result in a quantitative conversion into creatine. This result led the authors to formulate a reaction whereby the total guanido group of arginine is utilized for that of creatine, and they revived the old theory of Czernecki (23), according to which guanidoacetic acid is formed by β-oxidation of arginine (II). In later experiments by Davenport, Fisher, and Wilhelmi (24) it was found that the methyl group was derived from glycolic acid, which might originate from glycine. This theory conforms neither with the results of Borsook and Dubnoff (12), and du Vigneaud and collaborators (13), on the rôle of methionine, nor with the present finding that glycine is employed for the formation of the "sarcosine part" of creatine.

In 1927 Bergmann and Zervas (25) performed in vitro experiments which suggested that the amidine group and not the total guanido group of arginine may be employed for creatine formation. Triacetylanhydroarginine when treated with water is split into urea and acetylanhydroornithine (acetylaminopiperidine). When
the reaction is carried out in a water-free medium in the presence of glycine ester or sarcosine ester, the amidine group is not liberated as urea but is transferred to the other component to form

\[
\begin{align*}
\text{CH}_2\text{COOC}_2\text{H}_5 & \quad \text{CH}_2\text{COOC}_2\text{H}_5 \\
\text{NH}_2 & \quad \text{NH} \\
+ & \\
\text{HN-AC} & \quad \text{C=\text{N-AC}} \\
\text{C=\text{N-AC}} & \\
\text{N} & \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{HC-NHAc} & \quad \text{HC-NHAc} \\
\text{C=O} & \quad \text{C=O}
\end{align*}
\]

acetylguanidoacetic acid ester or acetylcreatine ester (III). In order to test the occurrence of the biological analogue to this highly suggestive chemical reaction it was necessary to synthesize and feed an arginine preparation, the amidine group of which contained isotope.

*Synthesis of Isotopic Arginine*—We have prepared this compound by replacing the amidine group in normal arginine by an isotopic amidine group. The \(\alpha\)-amino group of \(l(+)\)-arginine was blocked by the preparation of \(\alpha\)-toluenesulfonyl-\(l(+)\)-arginine (26), and the latter converted by boiling with aqueous baryta into \(\alpha\)-toluenesulfoornithine (IV). This treatment resulted in partial racemization. As the two components were not easy to separate, resolution was postponed until a later stage of the syn-
thesis. The partially racemic mixture was treated with isotopic methylisourea, prepared from isotopic cyanamide and methyl alcohol. The racemic toluenesulfoarginine could now be separated from the \( l(+) \) compound because of the different solubilities of the components in water. The toluenesulfo group was removed by treatment with hot concentrated hydrochloric acid, and pure \( l(+) \)-arginine monohydrochloride was obtained via the flavianate.

The addition of a small amount of this compound to the stock diet of rats resulted in the formation of creatine with isotope mainly in the amidine group. The result is thus qualitatively the same as that after feeding ammonia or \( \alpha \)-amino acids (except glycine). Isotopic arginine, however, is by far more effective.

This result in conjunction with the others leads to the conclusion that the amidine group of arginine has been employed for creatine

In our formula we have designated both nitrogen atoms of the amidine group as isotopic. This is in accordance with our formulation of isotopic creatine (11).
formation. The finding is well in accord with those obtained after feeding isotopic ammonia and $\alpha$-amino acids. As already stated, administration of these compounds always results in the formation of arginine with isotope in its amidine group. If the amidine group of arginine is transferred in the process of creatine formation, any nitrogen-yielding isotopic substance must indirectly result in the presence of isotopic creatine.

Our experiments give no evidence for the utilization of the entire guanido group of arginine for creatine formation. It has been shown that the substituted nitrogen atom of creatine is derived from glycine. If the whole guanido group of arginine had been involved in creatine formation, the nitrogen from glycine should have reached the sarcosine part of the creatine molecule via the $\delta$-amino group of ornithine. There is no evidence in the literature for the occurrence of such a process, which can also be excluded on the basis of former work with isotopes (27). When isotopic glycine was given, the nitrogen of the ornithine in the liver arginine contained only traces of marked nitrogen.

These observations on the rôle of arginine are in complete agreement with the recent results published in a preliminary note$^6$ by Borsook and Dubnoff (8). The authors had previously shown (12) the value of tissue slices for the investigation of creatine synthesis. They have now found kidney slices, in contrast to liver slices, rapidly to form guanidoacetic acid from arginine and glycine. The guanidoacetic acid was identified by isolation. These experiments thus also represent glycine and arginine as precursors of creatine. The finding was interpreted by the same chemical reaction; namely, by a shift of the amidine group.$^7$

Histidine—The imidazole ring has been claimed, on the basis of balance experiments, to be a creatine precursor (28). We have not carried out direct conversion experiments by feeding isotopic histidine, but can offer indirect evidence against the theory that

$^6$ This note on the action of arginine in tissue slices appeared almost simultaneously with our preliminary note (2) on isotopic arginine.

$^7$ Borsook and Dubnoff (8) have proposed the term “transamidination.” This term is coined according to “transamination,” which is a reversible reaction. The work with isotopes has as yet given no indication that the shift of the amidine group is reversible. “Transamidination” might thus be misleading, and we shall refer to it as “amidine shift.”
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this compound is involved in creatine formation. If the imidazole ring were the ultimate source or an intermediate, the animals that have formed isotopic creatine should also contain histidine with isotope in the imidazole ring. Arginine and glycine, both of which are intermediates in creatine formation, were always found to contain isotope when isolated from such animals.

The imidazole ring of histidine does not take up nitrogen from ammonia or \( \alpha \)-amino acids (29). Rats do not seem to have the ability to form this group, a finding in agreement with the statement that histidine is an indispensable amino acid for rats (30). However, the same animals did form isotopic creatine. The imidazole ring of histidine thus could not have been an intermediate.8

Urea—This is considered by Beard and Pizzolato (32) to be one of the most potent precursors of creatine. Urea with a high isotope concentration (15 atom per cent excess) was prepared in good yields and without nitrogenous side products by the action of ammonia (N\(^{15}\)) with diphenyl carbonate9 according to Hentschel (33).

The creatine isolated from the animals that had received this urea had only normal nitrogen. Urea is not a creatine precursor.10

8 Similar reasoning applies to the theory according to which uric acid (31) is a creatine precursor. We have isolated allantoin from the urine of the rats (unpublished) that had received isotopic arginine. The allantoin, which is the oxidation product of uric acid, had no appreciable amounts of isotope, while the creatine contained considerable quantities of marked nitrogen.

9 The authors are indebted to Dr. DeWitt Stetten, Jr., for help with the synthesis. When the procedure of Hentschel, worked out for 5 kilo lots, was repeated with 10 gm. samples of diphenyl carbonate, the reaction went extremely slowly and almost no urea was formed. When a small amount of copper powder was added to the melt, the reaction proceeded at a rapid rate and the yields of urea were satisfactory. The synthesis of Hentschel is thus catalyzed by copper, and it is probable that Hentschel in 1884, at a time when large glass or porcelain vessels were not easily obtainable, carried out the procedure in a copper bulb.

10 In an experiment, carried out for other purposes and not included in the tables, in which excessive doses of urea (100 mg. per day) were given to rats, the creatine had a very small isotope content (0.014 atom per cent excess); i.e., a minute amount of the nitrogen given as urea was present in the creatine. However, the feeding of such large quantities makes it difficult to interpret the biological findings. Part of the urea might have been degraded in the intestinal tract to ammonia, and the ammonia might
Choline—This compound has been considered by Riesser (34) to be a creatine precursor. Its metabolism is known, from recent reports (35–37), to be intimately connected with the metabolism of methionine, which yields its methyl group for creatine formation (12, 13). While choline is stated by Borsook and Dubnoff (12) not to yield its methyl group directly to guanidoacetic acid, it might do so, at least indirectly, via methionine.

This paper is not concerned with the transfer of methyl groups; our results with isotopic nitrogen cannot reveal their fate. According to the schemes proposed by Riesser, however, the nitrogen of choline is also utilized for creatine formation. We have prepared choline containing the nitrogen isotope. Isotopic ethanolamine was synthesized by condensing isotopic potassium phthalimide with ethylene bromide (38) and hydrolysis of the product with alkali (39). The base was converted into choline by methylation with dimethyl sulfate.

The creatine isolated after administration of this choline preparation had an isotope content only slightly above the error of the analytical procedure. The choline nitrogen is thus not employed directly for biological creatine synthesis.

Sarcosine—By treatment with alkali creatine and creatinine are degraded into ammonia, carbon dioxide, and sarcosine. The function of the latter as a creatine precursor has frequently been discussed. Like almost all other compounds, but unlike guanidoacetic acid, its administration does not increase creatine output (40).

Sarcosine has recently been shown by Abbott and Lewis (41) to be able to replace glycine as a precursor of hippuric acid, indicating its rapid conversion to glycine. A similar result has been obtained with isotopes (42). The feeding of isotopic sarcosine to rats is followed by almost the same distribution of isotope among the various amino acids of the proteins as is found when glycine is given. As the glycine of the proteins of the animals has a very high isotope content, demethylation of the sarcosine to glycine must have proceeded at a very rapid rate.

The creatine isolated after sarcosine feeding had an isotope have entered the creatine via the amidine group of arginine. Even if the result of this experiment were accepted as showing the utilization of urea for creatine formation (which we are not inclined to do), urea would still have to be considered as the most ineffective of the established precursors.
content slightly lower than that observed after the feeding of an equivalent amount of glycine. Sarcosine is therefore less effective than it should be if it represented one of the intermediate stages in creatine synthesis between glycine and creatine. The formation of isotopic creatine from isotopic sarcosine is thus only additional evidence of the rapid biological demethylation of sarcosine.

**Relative Efficiency of Nitrogenous Compounds As Creatine Precursors**

The efficiency of an isotopic compound as a creatine precursor is indicated in our experiments by the $N^{15}$ content in the isolated

### Table I

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Isotope content</th>
<th>Total nitrogen administered per day</th>
<th>Duration of experiment</th>
<th>Isotope content in</th>
<th>Total creatine or creatine</th>
<th>Amidine group</th>
<th>Sarcosine group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>atom per cent excess</td>
<td>mg.</td>
<td>days</td>
<td>atom per cent excess</td>
<td>atom per cent excess</td>
<td>atom per cent excess</td>
<td></td>
</tr>
<tr>
<td>dl-Tyrosine</td>
<td>2.00</td>
<td>14.4</td>
<td>10</td>
<td>0.001</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l(-)-Leucine</td>
<td>6.50</td>
<td>23</td>
<td>3</td>
<td>0.009</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>dl</em>-Glutamic acid</td>
<td>4.5</td>
<td>25</td>
<td>3</td>
<td>0.008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium citrate</td>
<td>2.00</td>
<td>26.4</td>
<td>9</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; + 300 mg. benzoic acid</td>
<td>1.21</td>
<td>72</td>
<td>6</td>
<td>0.025</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium citrate on protein-low diet*</td>
<td>1.21</td>
<td>180</td>
<td>5</td>
<td>0.036</td>
<td>0.044</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>4.5</td>
<td>23</td>
<td>3</td>
<td>0.080</td>
<td>0.000</td>
<td>0.070</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>4.5</td>
<td>25</td>
<td>6</td>
<td>0.078</td>
<td>0.016</td>
<td>0.205†</td>
<td></td>
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<tr>
<td>Arginine</td>
<td>4.5</td>
<td>23</td>
<td>3</td>
<td>0.225</td>
<td>0.049</td>
<td>0.515</td>
<td></td>
</tr>
<tr>
<td>Sarcosine</td>
<td>4.5</td>
<td>23</td>
<td>3</td>
<td>0.055</td>
<td>0.016</td>
<td>0.133†</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>8.3</td>
<td>23</td>
<td>3</td>
<td>0.108</td>
<td>0.130</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Guanidoacetic acid</td>
<td>1.5</td>
<td>4.2</td>
<td>6</td>
<td>0.128</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>1.5</td>
<td>4.5</td>
<td>6</td>
<td>0.528</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydantoic acid*</td>
<td>2.25</td>
<td>6</td>
<td>6</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylhydantoic acid*</td>
<td>2.25</td>
<td>5.2</td>
<td>6</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>15.2</td>
<td>23</td>
<td>3</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline*</td>
<td>2.00</td>
<td>19</td>
<td>3</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Immature rats.
† The value was not determined but calculated.
creatine or in its two nitrogenous groupings. The isotope content in the creatine samples isolated (Table I) is dependent upon a variety of factors, which include (1) the rate of creatine formation in the animal, (2) the duration of the experiment, and (3) the isotope concentration (in atom per cent N₁⁸ excess) of the compound administered.

**TABLE II**

<table>
<thead>
<tr>
<th>Material administered</th>
<th>Fraction of N derived from test substance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Of total creatine</td>
</tr>
<tr>
<td></td>
<td>per cent</td>
</tr>
<tr>
<td>dl-Tyrosine</td>
<td>0</td>
</tr>
<tr>
<td>l-Leucine</td>
<td>2.3</td>
</tr>
<tr>
<td>dl-Glutamic acid</td>
<td>2.3</td>
</tr>
<tr>
<td>Ammonia*</td>
<td>11.1</td>
</tr>
<tr>
<td>Glycine*</td>
<td>14.4</td>
</tr>
<tr>
<td>Sarcosine</td>
<td>7.7</td>
</tr>
<tr>
<td>Arginine</td>
<td>10.2</td>
</tr>
<tr>
<td>Hydantoic acid</td>
<td>22.0</td>
</tr>
<tr>
<td>Methylhydantoic acid</td>
<td>0</td>
</tr>
<tr>
<td>Urea</td>
<td>0</td>
</tr>
<tr>
<td>Choline</td>
<td>0</td>
</tr>
</tbody>
</table>

Guanidoacetic acid is not included in the table, as its administration is known to result in extra creatine formation. The efficiency of this compound calculated by employing the same factor would be 66.7 per cent.

* Only those experiments were considered in which adult rats were employed.

The conditions varied widely from experiment to experiment. The periods varied from 3 to 10 days, and the isotope concentrations in the administered compounds varied from 1.2 to 15.2 atom per cent. The rate of creatine formation in adult animals was probably constant within narrow limits except when guanidoacetic acid was given.

In order to compare the results of the various experiments the values obtained (Table I) have to be reduced to the same basis. In Table II are calculated for each experiment the fractions of the
newly formed creatine (or its two groupings) which had been derived from the nitrogen of the test substance. The assumption was made on the basis of our previous determination (11) that in each experiment 2 per cent of the creatine of the adult animals was newly formed per day.

The values in Table II were calculated by the following formula from the values given in Table I

$$\frac{b \times 100}{a \times t \times 0.02}$$

where $a =$ the isotope concentration (in atom per cent) of the material administered, $b =$ the isotope concentration (in atom per cent) in creatine (or its groupings), and $t =$ days of experiment.

Table II indicates the efficiency of each compound as a creatine former. 26 per cent of the nitrogen in the sarcosine group of creatine was derived from the isotopic glycine and about the same fraction of the amidine nitrogen was derived from the amidine group of the isotopic arginine. None of the values could have reached 100 per cent, even if the nitrogen of glycine and arginine were the only sources for creatine synthesis. The isotopic compounds added to the diet had rapidly merged with the same non-isotopic compounds derived from the dietary casein and the proteins of the animals. The nitrogen that was utilized must thus have been a mixture of isotopic and non-isotopic material. If glycine and arginine had been the only sources, the values found would indicate that the isotopic glycine had been mixed in the metabolism with 4 times its weight of ordinary glycine, and the isotopic arginine with about 4 times its weight of ordinary arginine before either had reached the site of creatine formation. On the basis of recent experiments which demonstrated the rapidity with which dietary and tissue constituents interact, such an assumption seems reasonable. However, as we do not know exactly the extent of this mixing process, we cannot state definitely that glycine and arginine are the only sources for creatine nitrogen. As the other compounds investigated showed little or no effect, the nitrogen of glycine and that of the amidine group of arginine obviously represent the main sources of the nitrogen of creatine.
The present results, in conjunction with those on the methyl transfer (12, 13), seem to establish the immediate biological origin of every part of the creatine molecule (V).

\[
\begin{align*}
\text{From proteins} & \quad \text{From arginine} & \quad \text{From methionine} \\
\text{NH}_2 & \quad \text{NH}_2 & \quad \text{NH}_2 \\
\text{CH}_2\text{COOH} & \quad \text{C}==\text{NH} & \quad \text{C}==\text{NH} \\
& + \text{H}_2\text{N}--\text{C}==\text{NH} & + \text{CH}_3 \\
\text{NH} & \quad \text{NH} & \quad \text{N}--\text{CH}_2 \\
\text{CH}_2\text{COOH} & \quad \text{CH}_2\text{COOH} & \quad \text{CH}_2\text{COOH}
\end{align*}
\]

(V)

Not only the precursors but also the sequence of the condensation reaction seems to be established. Methylation cannot have occurred before the condensation of glycine with the amidine group, as guanidoacetic acid is an intermediate, while sarcosine is not.

Creatine As a Measure of Endogenous Metabolism

Folin (43), in his classical study of creatine metabolism, put forward evidence according to which creatinine excretion is independent not only of protein intake but also of creatine. He contended that creatinine despite its chemical relation to creatine is metabolically independent of it. He assumed that creatinine, like urea, is a metabolic waste product but suggested that both originate from different types of protein catabolism, one of which (resulting in creatinine, etc.) is constant, while the other (resulting in urea and ammonia) is dependent upon the amount of protein intake. In accordance with the concept, prevalent at that time, that the food material, including amino acids, is directly degraded for energy requirements, whereas the structural body components are mainly stationary, he attributed urea formation to the catabolism of food constituents (variable or exogenous metabolism) and creatinine to that of the tissue proteins (constant or endogenous metabolism). Creatinine formation, according to Folin, probably represents "an essential part of the activity which distinguishes living cells from dead ones."
The interpretation of some of the experimental results on which Folin's hypothesis is based has since been found to be incorrect. Creatinine excretion is not independent of creatine intake, as the feeding of creatine is followed by a delayed extra excretion of creatinine (44).

Recently (15) the normal creatinine of the urine has been demonstrated to be derived directly from body creatine and its nitrogen must be regarded as representative of that of the tissue creatine. Both compounds must thus have identical sources.

The present study has shown that a considerable fraction of the creatine nitrogen (and therefore also of the creatinine nitrogen) is provided by normal food constituents; i.e., glycine and arginine. As much as 25 to 30 per cent of the nitrogen in the newly formed creatine had been derived from the isotopic compounds added to the casein-containing stock diet, and an additional part of the creatine nitrogen must have been derived from the same, non-isotopic, amino acid in the dietary proteins. The remainder of the creatine nitrogen must have been supplied by the arginine and glycine of the tissues. Creatine and creatinine are thus constantly formed from food as well as tissue material. The present findings on the sources of creatine (and therefore also of creatinine) provide additional evidence against a concept of two separate types of catabolism.\textsuperscript{11}

**EXPERIMENTAL**

*Isotopic guanidoacetic acid* was prepared according to Strecker (14). To 0.75 gm. of glycine (4.5 atom per cent N\textsuperscript{15} excess) in 5 cc. of water were added 0.84 gm. of non-isotopic cyanamide and 3

\textsuperscript{11} In a recent paper by Burroughs, Burroughs, and Mitchell (45) the theory of Folin, including the concept of creatinine as a measure of endogenous metabolism, is upheld and the findings of this laboratory on the rapid chemical interactions of dietary amino acids and tissue proteins are given a new interpretation: The chemical activity of the body proteins is restricted to the "dispensable reserve proteins" and does not involve the "fixed" proteins. According to this concept one would thus have to distinguish between the catabolism of food and dispensable reserve proteins on the one hand and that of specific cell constituents on the other. Acceptance of this interpretation would compel, as a corollary of the present findings, the view that creatine and creatinine are formed from food and "dispensable reserve proteins." This would lead to the hardly acceptable conclusion that they are derived from "exogenous" metabolism only.
drops of concentrated ammonia. The mixture was kept at room temperature for 3 days. The crystalline precipitate was filtered off and recrystallized from a large volume of water. The yield was 0.75 gm.

\[
\text{N (Kjeldahl) calculated, 35.9; found, 36.0} \\
\text{N}^{15} \text{ calculated, 1.50 atom per cent excess; found, 1.50}
\]

*Isotopic hydantoic acid* was prepared according to West (16). 0.525 gm. of glycine (4.5 atom per cent N\(^{15}\) excess) and 0.61 gm. of non-isotopic potassium cyanate were dissolved in 20 cc. of water and the mixture heated on the steam bath for 1 hour. The solution was filtered, cooled, and made acid to Congo red by addition of concentrated hydrochloric acid. The crystalline precipitate was recrystallized from a small volume of water. The yield was 0.50 gm.

\[
\text{N (Kjeldahl) calculated, 23.7; found, 23.8} \\
\text{N}^{15} \text{ calculated, 2.25 atom per cent excess; found, 2.26}
\]

*Isotopic N-methylhydantoic acid* was prepared according to Salkowski (17). 0.446 gm. of sarcosine (4.5 atom per cent N\(^{15}\) excess) and 0.41 gm. of normal potassium cyanate in 7 cc. of water were heated together on the steam bath for 5 minutes. The mixture was cooled, and acidified by addition of hydrochloric acid. The crystalline precipitate was filtered off and recrystallized from a small volume of water. The yield was 0.33 gm.

\[
\text{N (Kjeldahl) calculated, 21.2; found, 21.2} \\
\text{N}^{15} \text{ calculated, 2.25 atom per cent excess; found, 2.25}
\]

**Preparation of Isotopic Arginine**

\(\alpha-p\)-Toluenesulfonylornithine—\(\alpha-p\)-Toluenesulfonylarginine was prepared from \(l(+)-\)arginine monohydrochloride (26). The compound had a rotation of \([\alpha]_{D}^{22} = -15.0^\circ\) (3.3 per cent in 15 per cent hydrochloric acid). 43 gm. were dissolved in a solution containing 350 gm. of crystalline barium hydroxide in 2100 cc. of water and boiled under a reflux for 21 hours, when no more ammonia was liberated. The barium was removed by addition of exactly the necessary amount of sulfuric acid, and filtrate and washings were brought to dryness in *vacuo*. The dry residue was extracted several times with 70 per cent acetone and the combined
extracts concentrated to a small volume. The crystals obtained by cooling were recrystallized from 85 per cent ethanol. The yield was 26 gm. (79 per cent of the calculated amount) of toluenesulfonylornithine, m. p. 207°.

N (Kjeldahl) calculated, 9.8; found, 9.7
S calculated, 11.2; found, 11.0
\([\alpha]_D^{25} = -3.15^\circ (6.5\% \text{ in } 15\% \text{ hydrochloric acid})\)

The product so obtained had been partially racemized. It has a lower rotation than a sample of toluenesulfonylornithine which was obtained by the action of arginase upon toluenesulfonylarginine. This latter substance also melted at 207°, but had a rotation of \([\alpha]_D^{25} = -5.5^\circ (2 \text{ per cent in } 15 \text{ per cent hydrochloric acid})\).12

**Isotopic O-Methylisourea**—Isotopic cyanamide was prepared from non-isotopic cyanogen bromide and isotopic ammonia (34.0 atom per cent N\(^{15}\) excess) as described before (11). The isotopic cyanamide was converted into O-methylisourea hydrochloride according to Stieglitz (46), and the free base was obtained from the hydrochloride according to Kapfhammer and Mueller (47).

**Isotopic Toluenesulfonyl-L(-)-Arginine**—The solution of 2.8 gm. of O-methylisourea in 25 cc. of methanol was added to 7.5 gm. of toluenesulfonylornithine, dissolved in a mixture of 75 cc. of water and 220 cc. of methanol. The procedure was thus similar to that of Kapfhammer (47) for the preparation of other guanido acids. The mixture was treated with 3 cc. of concentrated non-isotopic ammonia and kept in the ice box for a week. The crystalline precipitate weighed 4.7 gm. (Fraction A). On concentration of the filtrate a second crop, 2.5 gm. (Fraction B), was obtained. The total yield of crude toluenesulfonylarginine was 7.2 gm., or 82 per cent of the calculated amount.

Fraction A consisted mainly of the \(dl\) component, with a rotation of \([\alpha]_D^{24} = -1.8^\circ (4 \text{ per cent in } 15 \text{ per cent hydrochloric acid})\). Repeated crystallizations by dissolving in an equivalent amount

---

12 As we had obtained only a small amount (60 mg.) of this sample, the value of the rotation is not very precise. The enzymatic degradation of toluenesulfonylarginine is not practical, as it requires very large amounts of enzyme. We are indebted to Dr. M. Richards of this department for a sample of purified arginase.
of hydrochloric acid and neutralizing with sodium carbonate did not accomplish complete removal of the \(l\) isomer. After three crystallizations the rotation was \(\left[\alpha\right]_{D}^{24} = -0.7^\circ\) (4 per cent in 15 per cent hydrochloric acid). The compound started to decompose slowly at 265°. It is very slightly soluble in water.

Calculated, N 17.1, S 9.8; found, N 16.9, S 9.9

Fraction B melted at 256°. After one recrystallization from water it had a rotation of \(\left[\alpha\right]_{D}^{23} = -15.1^\circ\) (2 per cent in 15 per cent hydrochloric acid); i.e., the same rotation as that of toluenesulfonyl-\(l(+)\)-arginine prepared by direct toluenesulfonylation of \(l\)-arginine. Thus, the more water-soluble reaction product consisted of toluenesulfonyl-\(l(+)\)-arginine, whereas the less soluble fraction represented mainly the \(dl\) component.

Isotopic \(l(+)\)-Arginine Monohydrochloride—For the removal of the toluenesulfonyl group the \(l\) and the \(dl\) fractions were hydrolyzed separately by heating with concentrated hydrochloric acid in sealed tubes on the steam bath for 36 hours. The reaction mixture was brought to dryness in vacuo, the residue taken up in water, and a slight excess of flavianic acid was added. From 3.23 gm. of \(dl\)-toluenesulfonylarginine there were obtained 4.68 gm. of \(dl\)-arginine monoflavianate, corresponding to a yield of 96 per cent, and from 2.44 gm. of \(l\)-toluenesulfonylarginine 3.3 gm. of \(l\)-arginine monoflavianate, corresponding to a yield of 90 per cent.

Flavianic acid was removed from the \(l\) salt by suspension in concentrated hydrochloric acid and filtering off the flavianic acid. The filtrate was diluted with water, treated with norit, and brought to dryness in vacuo. The residue was taken up in 95 per cent ethanol and pyridine was added. The yield after recrystallization from water-ethanol was 1.20 gm. of \(l(+)\)-arginine monohydrochloride or 77 per cent of the amount calculated from the weight of toluenesulfonylarginine taken.

Calculated, C 34.1, H 7.2, N 26.7; found, C 34.0, H 7.0, N 26.9
\(\left[\alpha\right]_{D}^{23} = +24.9^\circ\) (1.2% in 15% hydrochloric acid) calculated for free arginine

In the course of the arginine synthesis the isotopic ammonia (34.0 atom per cent N\(^{15}\) excess) originally employed for the synthesis of O-methylisourea had been diluted with 3 atoms of
Biological Precursors of Creatine

non-isotopic nitrogen (a) by 1 atom from the non-isotopic cyanogen bromide and (b) by 2 atoms from the non-isotopic toluenesulfonyl-ornithine. The isotope concentration in the arginine should therefore be 34.0/4 = 8.5 per cent. The concentration found was 8.3 atom per cent N\textsuperscript{15} excess.

Isotopic Ethanolamine—Bromoethylphthalimide was prepared as described in “Organic syntheses” (38) from 37 gm. of isotopic potassium phthalimide (2.0 atom per cent N\textsuperscript{15} excess) and 90 gm. of ethylene dibromide. The yield of bromoethylphthalimide was 33.8 gm. or 67 per cent of the calculated amount. This was subjected to alkaline hydrolysis with 30 per cent potassium hydroxide solution according to Putokhin (39). The ethanolamine was distilled from the reaction mixture into an excess of dilute hydrochloric acid. Ethanolamine hydrochloride was isolated from its alcoholic solution by precipitation with ether. The yield was 9.23 gm., or 71 per cent.

Calculated. N (Kjeldahl) 14.4, (amino) 14.4
Found. " " 14.6, " 14.3
N\textsuperscript{15} calculated, 2.0 atom per cent excess; found, 2.0

Isotopic Choline—To a cooled solution of 3.9 gm. of ethanolamine hydrochloride (2.0 atom per cent N\textsuperscript{15} excess) in 170 cc. of 5 per cent potassium hydroxide solution, 25.5 gm. of dimethyl sulfate were gradually added with stirring. The stirring was continued for 1 hour and the filtered solution made acid to Congo red with hydrochloric acid. On the addition of 15 gm. of gold chloride, 14.2 gm. of choline gold chloride were obtained, or 80 per cent of the calculated amount. The substance melted at 268° with decomposition.

N (Kjeldahl) calculated, 3.16; found, 3.17
N\textsuperscript{15} calculated, 2.0 atom per cent excess; found, 2.0

Isotopic Urea—The compound was prepared according to Hentschel (33). 2.72 gm. of diphenyl carbonate and 50 mg. of copper dust were placed in a steam-jacketed shaking flask. The flask, while being heated to 100°, was evacuated and connected with a vessel in which ammonia was generated from 1.70 gm. of isotopic ammonium chloride (15.3 atom per cent N\textsuperscript{15} excess). The uptake of ammonia was complete after 30 minutes. The
reaction mixture was taken up in water and extracted twice with chloroform. The aqueous solution was treated with norit, filtered, and brought to dryness in vacuo. The residue was taken up in a small volume of ethanol and precipitated with benzene. The crystalline precipitate of urea was further purified by extraction from a thimble with acetone in a Soxhlet apparatus. The urea crystallized from acetone weighed 0.44 gm., or 58 per cent of the calculated amount. The compound melted at 132-133°.

\[
\begin{align*}
N \text{ (Kjeldahl)} & \text{ calculated, 47.0; found, 47.0} \\
N^{15} & \text{ calculated, 15.3 atom per cent excess; found, 15.2}
\end{align*}
\]

**Feeding Experiments**

The experimental conditions employed in the feeding of the following substances have been described before: ammonia (21, 22), glycine (27), sarcosine (42), \textit{dI-tyrosine} (48), \textit{l-leucine} (49). In all these experiments muscle creatine and urinary creatinine were isolated by the methods previously reported (15). The analytical values are given in Table I. All animals received the same stock diet (15).

**Glycine Feeding to Immature Rats**—Two growing rats, having a combined weight of 185 gm., received 25 mg. of glycine nitrogen (4.5 atom per cent N\textsubscript{15} excess) each day for 6 days, when they were killed. At the end of the feeding period the animals had a combined weight of 246 gm. Muscle creatine was isolated and degraded into ammonia and sarcosine by baryta.

**Guanidoacetic Acid Feeding I**—Two adult rats, having a combined weight of 568 gm., were given 11.7 mg. of guanidoacetic acid (1.5 atom per cent N\textsubscript{15} excess) per day per rat for 6 days. The animals were then killed and the muscle creatine isolated.

**Guanidoacetic Acid Feeding II**—Two growing rats, having a combined weight of 179 gm., received 25 mg. of guanidoacetic acid (1.5 atom per cent N\textsubscript{15} excess) per rat per day for 6 days. The animals gained a total of 55 gm. After the animals had been killed, muscle creatine was isolated. The isotope concentration was also determined in the total urinary nitrogen excreted during the feeding period as well as in the urinary urea.

\[
\begin{align*}
\text{Total urinary nitrogen, } & 0.116 \text{ atom per cent } N^{15} \text{ excess} \\
\text{Urea, } & 0.004 \text{ atom per cent } N^{15} \text{ excess}
\end{align*}
\]
The fact that a considerable amount of the administered isotope was excreted, but was not present in the urea, suggests that part of the guanidoacetic acid was excreted unchanged. This is also indicated by the strongly positive Sakaguchi reaction in the urines. These results are in agreement with those of Jaffe (50) who showed that an excess of guanidoacetic acid is not metabolized but excreted as such.

**Hydantoic Acid Feeding**—One growing rat, weighing 92 gm., received 25 mg. of hydantoic acid (2.25 atom per cent N\(^{15}\) excess) prepared from isotopic glycine (4.5 atom per cent N\(^{15}\) excess) and non-isotopic potassium cyanate, per day for 6 days, when it was killed. The animal had gained 24 gm. Muscle creatine was isolated and found to contain no excess of isotope.

**Methylhydantoic Acid Feeding**—One growing rat, weighing 102 gm., received 25 mg. of methylhydantoic acid (2.25 atom per cent N\(^{15}\) excess) prepared from isotopic sarcosine and non-isotopic potassium cyanate, per day for 6 days, when it was killed. The animal had gained 20 gm. The isolated muscle creatine did not contain excess of isotope.

**Choline Feeding**—Three immature rats, weighing 199 gm., received 19 mg. of choline nitrogen (2.0 atom per cent N\(^{15}\) excess) per day per rat for 3 days. The choline was obtained from choline aurichloride by removing the gold with hydrogen sulfide. At the end of the feeding period the animals weighed 208 gm. They were killed and the muscle creatine isolated.

**Urea Feeding**—Two adult rats, having a combined weight of 557 gm., received 23 mg. of urea nitrogen (15.2 atom per cent N\(^{15}\) excess) per day per rat for 3 days. Muscle creatine was isolated and found to contain no excess of isotope.

**Feeding of L(+)-Arginine**—Two adult rats, having a combined weight of 646 gm., received 23 mg. of arginine nitrogen (8.3 atom per cent N\(^{15}\) excess) per rat per day for 3 days. The animals were then killed and creatine was isolated from the muscles. The creatine sample was degraded with barium hydroxide and isotope analysis was made of the ammonia and the sarcosine. The values are given in Table I.

**SUMMARY**

1. The formation of creatine in normal rats was investigated by adding to their ordinary stock diet twelve different compounds...
containing the nitrogen isotope. The substances tested were (1) ammonia, (2) dl-tyrosine, (3) l(-)-leucine, (4) dl-glutamic acid, (5) glycine, (6) l(+)-arginine, (7) sarcosine, (8) guanido-acetic acid, (9) hydantoic acid, (10) methylhydantoic acid, (11) choline, (12) urea. The preparation of Compounds 6 and 8 to 12 is described. At the end of the feeding period creatine samples were isolated from tissues of the animals and their isotopic content was determined.

2. In order to locate the position of the isotopic nitrogen among the nitrogen atoms of the isolated creatine, some of the samples were degraded with barium hydroxide, whereby the nitrogen of the amidine group was recovered as ammonia, and the remaining nitrogen as sarcosine.

3. Urea, hydantoic acid, methylhydantoic acid, and choline are not creatine precursors. Creatine isolated after feeding these compounds did not contain the isotope marker.

4. Arginine and glycine are the only natural amino acids investigated so far which are potent precursors of creatine; their feeding results in the formation of creatine with a high isotope content. Each of the two compounds supplies nitrogen to different parts of the creatine molecule; glycine is utilized for the sarcosine moiety; the amidine group of arginine supplies the amidine group of creatine.

5. After the feeding of isotopic ammonia and of the isotopic amino acids tyrosine, leucine, and glutamic acid, the creatine has only a very low concentration of isotope. The ammonia, when given in very large amounts, yields creatine with an isotope concentration high enough for degradation studies. Most of the isotope was found to be present in the amidine part of the creatine; i.e., the effect of these compounds on creatine formation is qualitatively the same as that of feeding arginine with an isotopic amidine group. These substances are only indirect creatine precursors: they yield nitrogen to the amidine group of the arginine of proteins which in turn is shifted to glycine to form guanidoacetic acid. A small amount of isotopic glycine, formed with the nitrogen from these compounds, is also employed for creatine synthesis.

6. Interpretation of the quantitative data reveals that in the present experiments a minimum of 26 per cent of the amidine nitrogen of the newly formed creatine was derived from that of the
added arginine, and 26 per cent of sarcosine nitrogen of the creatine was derived from the added glycine.

7. The hypothesis that guanidoacetic acid is an intermediate in creatine formation is corroborated by the finding that the feeding of isotopic guanidoacetic acid results in creatine with a very high isotope content. Guanidoacetic acid is the most potent creatine precursor investigated so far.

8. Sarcosine, while almost as effective as a precursor of creatine as is glycine, cannot be considered as an intermediate. Its effectiveness is due to its rapid demethylation to glycine.

9. The present findings, in conjunction with the recent observation of other laboratories, according to which the methyl group of creatine is supplied by methionine, establish the immediate biological origin of all parts of the creatine molecule, as well as the sequence of their condensations. Glycine reacts with the amidine group of arginine to form guanidoacetic acid, and this is methylated to form creatine by shift of the methyl group from methionine.

10. The finding that the newly formed creatine (and creatinine) molecules acquire their parts from food as well as from tissue components is taken as further evidence against the concept of two independent (exogenous and endogenous) types of catabolism.

Addendum

The present findings on the sources of creatine cannot be reconciled with the experimental results or with the theory of Beard et al. (32, 51, 52). According to Beard and Pizzolato (32) the injection of amino acids (and some other nitrogenous compounds) into rats results in a large increase of muscle creatine. These findings in conjunction with some others were interpreted as showing that the natural amino acids are converted into glycine and urea, both of which condense to form guanidoacetic acid, which in turn is methylated by another molecule of glycine to form creatine. The experiments could not be reproduced by other investigators (53), and are also in conflict with the general belief that the rate of creatine formation is almost independent of the amount of dietary amino acids.

Beard and Pizzolato (32) did not compute the actual amounts of creatine formed by their rats from injected amino acids. We
K. Bloch and R. Schoenheimer have calculated from their values how much of the nitrogen of the administered amino acid must have been utilized for creatine formation. This was based on the following data. The authors state the amount of amino acids in mg. administered and the resulting increase of muscle creatine in per cent of preformed creatine. The weights of the animals were given as 100 to 250 gm., and they assumed in their own calculations that the average weight of their rats was 200 gm. This value was also taken for our calculations. They consider such rats to contain 80 gm. of muscle tissue, and the rat muscle was found by them to contain 0.40 per cent creatine. The average rat employed for their studies thus contained a total of 320 mg. of muscle creatine, or 102 mg. of creatine nitrogen. If, as was found by these authors, 200 mg. of aminobutyric acid increase the content of muscle creatine by 38

TABLE III

Utilization of Amino Acids for Creatine Formation According to Beard and Pizzolato (32)

<table>
<thead>
<tr>
<th>Compound injected</th>
<th>N injected (mg.)</th>
<th>Increase of muscle creatine observed (3)</th>
<th>Increase of creatine N calculated (4)</th>
<th>Utilization of injected N for creatine formation (5)</th>
<th>Values calculated for 1 nitrogen atom in creatine (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a-Aminobutyric acid, 200</td>
<td>27</td>
<td>38</td>
<td>39</td>
<td>143</td>
<td>48</td>
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<td>a-Aminocaproic acid, 200</td>
<td>21</td>
<td>45</td>
<td>46</td>
<td>216</td>
<td>72</td>
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<td>a-Aminovaleric acid, 200</td>
<td>24</td>
<td>52</td>
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<td>75</td>
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<td>22</td>
<td>23</td>
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<td>23</td>
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<td>Glycine, 200</td>
<td>37</td>
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<td>Urea, 300</td>
<td>140</td>
<td>29</td>
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<td>21</td>
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</table>
per cent, this means that 27 mg. of injected amino nitrogen have produced 39 mg. of creatine nitrogen, or 143 per cent. The results of some of the calculations are given in Column 5 of Table III. We have considered in each series only those positive experiments in which the smallest amount of compound was injected.

According to these values most compounds have yielded much more than 100 per cent of their nitrogen for creatine formation. It is interesting to note that urea, glycine, and guanidoacetic acid are according to these calculations relatively ineffective. If only 1 nitrogen atom of the creatine be assumed to have had its origin in the injected compound, the amount of nitrogen utilized for creatine formation would be only one-third of these values. The resulting figures (Column 6) are still so high as to suggest that creatine formation (and not urea formation, as is generally believed) is one of the main end-products of the metabolism of most amino acids.

Beard and Pizzolato (54) still hold to their contention that creatine is not converted into creatinine, but that the reverse process occurs; i.e., formation of creatine from creatinine. They base their claim on experiments of Beard and Jacob (55), who showed that injection of as little as 10 mg. of creatinine into rats results in an increase of muscle creatine by 19 per cent. This, according to the above calculation, would correspond to 60.8 mg. of creatine, or 520 per cent of the theory.

BIBLIOGRAPHY

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THE BIOLOGICAL PRECURSORS OF CREATINE
Konrad Bloch and Rudolf Schoenheimer


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