UREA AS A SOURCE OF NITROGEN FOR THE BIOSYNTHESIS OF AMINO ACIDS*

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Urea has long been recognized as the principal end product of nitrogenous metabolism in mammals. In recent years, the possibility that monogastic animals might be able to decompose this substance, when administered preformed, has stimulated considerable interest. The presence of radioactive carbon dioxide in the expired air after the injection of urea labeled with C\textsuperscript{14} indicates that decomposition of this compound actually occurs in the mouse (2) and the rat (3), and to a slight extent in the cat (4). However, attempts to determine whether the rat, as a typical non-ruminant, can utilize the nitrogen of urea for synthetic purposes have yielded divergent conclusions. Thus, Kriss and Marcy (5) found that urea administered orally to immature rats receiving a presumably normal diet exerted no change in body weight and was almost quantitatively recovered as such in the urine and feces. Similar results have been reported by Bloch (6), following experiments upon mature rats. This investigator added urea which had been labeled with N\textsuperscript{15} to a diet containing casein, and observed the extent of excretion of the compound and the degree of incorporation of the isotope in certain tissue constituents. The findings led him to conclude that urea is devoid of any metabolic activity.

The above data are in striking contrast to those set forth in a previous paper from this laboratory by Rose, Smith, Womack, and Shane (7). The latter were investigating the types of compounds which might serve as sources of nitrogen for the synthesis of the non-essential amino acids. For this purpose, advantage was taken of the fact that the growth of weanling rats is markedly inhibited by restricting the dietary nitrogen to that present in the essential amino acids when each is furnished at the minimal level

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compatible with maximal growth (8–10). It should be recalled that these minimal values were established under conditions which provided an abundance of all amino acids other than the one under investigation (cf. (8)). Consequently, one would anticipate that a diet carrying the essential amino acids only, each at its minimal level, would be incapable of supporting normal gains in weight, since, under these circumstances, sufficient nitrogen would not be available for the synthesis of the non-essential acids. In other words, under the conditions specified, a shortage of nitrogen might become the limiting factor in the growth of the subjects. Experience showed this supposition to be correct; but of greater significance for the problem at hand was the disclosure that the growth of the animals could be conspicuously accelerated by the addition to the basal ration of any one of several nitrogenous compounds, notably ammonium salts, L-glutamic acid, glycine, and even urea. These findings, which have been confirmed elsewhere (11), appear to permit of only one reasonable interpretation, namely that the nitrogen of the dietary supplements was utilized in the synthesis of amino acids.

It should be emphasized that the diets employed in the experiments of Kriss and Marcy (5) and Bloch (6) were "normal" with respect to their nitrogen content, and may be presumed to have furnished all of the amino acids. On the other hand, as pointed out above, the growth tests conducted in this laboratory (7) involved the use of a ration which was devoid of the non-essential amino acids and carried only minimal quantities of the essentials. In view of this fundamental difference, it seems plausible to suspect that the character of the nitrogen intake may have been responsible for the divergence between our findings and those of others. To test this hypothesis, a comparison was made of the effects of adding urea to rations in which the nitrogen was supplied in the form of (a) casein, and (b) a mixture of the essential amino acids at their minimal levels. Furthermore, the urea was labeled with N\(^{15}\) in order thereby to permit direct measurements of the extent of incorporation of the isotope in the amino acids of the tissues. The conduct of the experiments and the results obtained are outlined below. The data serve to substantiate the conclusions reached by the growth technique and to provide a reasonable explanation for the negative findings of others.

**EXPERIMENTAL**

**Preparation of Labeled Urea**—The starting material in the preparation of labeled urea was ammonium nitrate containing 63.5 atom per cent excess of N\(^{15}\) in the ammonium ion.\(^1\) The salt was converted into ammonium chloride by treatment with strong alkali and distillation into an excess of

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\(^1\) Purchased from the Eastman Kodak Company, Rochester, New York.
standard hydrochloric acid. The apparatus used for this purpose was similar to that described by Schoenheimer and Ratner (12). During the distillation, a slow stream of washed nitrogen was passed through the system to facilitate the transport of the ammonia. The resulting solution of ammonium chloride was then treated with a slight excess of a suspension of freshly prepared silver cyanate. The flask containing the mixture was protected from light and agitated for 6 hours on a mechanical shaker. After removing the silver salts by filtration, the combined filtrate and washings were slowly evaporated to dryness over a steam cone to accomplish the Wöhler rearrangement of the isotopic ammonium cyanate into urea. Repeated extraction of the residue with 95 per cent ethanol removed the urea and left behind a grayish material derived from the slight excess of silver cyanate. The urea was purified by recrystallization from hot, absolute ethanol. Finally, it was recrystallized with an appropriate amount of previously analyzed unlabeled urea to yield a product containing the desired level of isotope. The preparation as used in the animal experiments contained 14.75 atom per cent excess of N\textsuperscript{15}. It melted sharply at 132–133\textdegree (uncorrected) and showed a total nitrogen content which agreed closely with the theoretical value.

\[ \text{CH}_2\text{N}_2\text{O}. \] Calculated, N 46.91; found, N 46.88\textsuperscript{4}

The purity of the labeled urea was further demonstrated by comparing its isotopic content with that of a sample of urea oxalate prepared from it.

**Feeding Experiments**—Six male, weanling rats of the same litter served as the experimental subjects. At the start of the feeding tests, they were quite uniform in size and had an average body weight of 45 gm. They were divided as equitably as possible into two groups of three rats each. Hereafter, they will be referred to as the animals of Group A, which received Diet A containing the essential amino acids, and the animals of Group C, which received Diet C containing casein. Each rat was housed in a separate cage, which was designed to prevent food scattering and to permit quantitative collection of the urine and feces.

The make-up of the basal diets is shown in Table I. As will be observed, Diet A contained amino acid Mixture XXVI, which was the mixture used by Rose et al. (7) in demonstrating the growth-stimulatory effect of added urea. Diet C was a normal ration. It carried 18 per cent of casein and 0.20 per cent of dl-methionine. In our experience, the addition of methionine improves the quality of casein and permits slightly better growth. Both diets were supplemented with suitable quantities of vitamins, as described elsewhere (cf. (10)).

Throughout the entire experiment, the rats of Group A were permitted

\[ ^2 \text{Values are corrected for 14.75 atom per cent excess of N}^{15}. \]
to consume food *ad libitum*. During the first 12 days, the basal ration (Diet A) alone was administered. At the expiration of this fore period, the labeled urea was incorporated in the ration in the proportion of 1.23 per cent and at the expense of an equal weight of dextrin. The labeled urea furnished 0.577 gm. of additional nitrogen per 100 gm. of Diet A. This is comparable to the nitrogen content (0.574 gm.) of the non-isotopic urea previously found to be effective in the stimulation of growth (7). A total of 176 gm. of Diet A was supplemented with labeled urea, and the

**Table I**

*Composition of Basal Diets*

<table>
<thead>
<tr>
<th>Component</th>
<th>Diet A</th>
<th>Diet C</th>
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</thead>
<tbody>
<tr>
<td>Amino acid Mixture XXVI*</td>
<td>8.82</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td></td>
<td>18.00</td>
</tr>
<tr>
<td>dL-Methionine</td>
<td>15.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>67.43</td>
<td>58.05</td>
</tr>
<tr>
<td>Dextrin</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Cellu flour</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Salt mixture†</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Corn oil</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Haliver oil‡</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Inositol</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Liver extract§</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

* Rose *et al.* (7).
† Jones and Foster (13).
‡ This contained 65,000 U. S. P. units of vitamin A and 13,000 U. S. P. units of vitamin D per gm.
§ Wilson's liver powder, 1:20.

The rats of Group C were allowed to consume the casein diet (Diet C) *ad libitum* for 8 days. As was to be anticipated, they made rapid gains in weight; consequently, the paired feeding technique was instituted on the 9th day and was continued to the end of the experiment. According to this procedure, the food intake of the animals of Group C was limited to the voluntary consumption of the rats of Group A. Since all animals were weighed and their food intakes determined at 4 day intervals, use of the excess N¹⁵ therein contained was 158.8 mg. The food was kept constantly before the animals of Group A until it was entirely consumed, care being taken to note the exact time when the last trace disappeared. This occurred in 7½ days.
paired feeding technique necessitated an extension of the fore period of Group C by 4 days in order that an accurate estimate of the consumption of Group A during the preceding 4 days might be established. After 16 days, 1.23 per cent of isotopic urea was incorporated in 176 gm. of Diet C, at the expense of dextrin, and the resulting supplemented ration was administered for exactly 7\(\frac{3}{4}\) days. To equalize, as nearly as possible, the conditions to which the two groups of animals were being subjected, the daily allotment of Diet C was furnished to each member of Group C in three equal portions at 8 hour intervals. Thus, during the period of urea supplementation, the rats of each group received exactly the same weight of food and of isotopic urea.

Beginning with the addition of urea to the diets, the urines were collected under toluene. At frequent intervals, the funnels through which the urines passed to the receiving vessels were washed down with a fine stream of 2 per cent boric acid solution. Each sample was strained through a wad of cotton prior to storing. At the conclusion of the feeding tests, the urines from the three animals of each group were combined and reserved at a low temperature for analysis. The feces were collected several times daily, covered with acidified ethanol, and evaporated to dryness. At the end of the period, the combined fecal material from the three rats of each group was ground to a fine powder and saved for analysis.

The animals were killed by decapitation immediately after they had completed the consumption of the supplemented diets. The gastrointestinal tracts were first excised. Formed pellets of fecal material were recovered and added to the appropriate samples previously collected. The remainder of the alimentary contents was removed by thorough washing and the carcasses of the three members of each group were combined for subsequent work. For the latter purpose, they were cut into small pieces, frozen, and passed through a meat grinder. Each of the resulting mixed tissues was homogenized in a Waring blender with two 500 ml. portions of 10 per cent trichloroacetic acid solution. After filtration, the residues were extracted twice with 300 ml. portions of acetone and repeatedly with ether. When dried in air, fluffy powders resulted, which consisted predominantly of the proteins and part of the inorganic components of the animals.

Isolation and Analytical Procedures—Portions of the carcass powders described above were used in ascertaining their total and isotopic nitrogen content. The methods employed will be outlined later. Larger samples were used in isolating individual amino acids. From each, one essential and several non-essential amino acids were procured. Urea was obtained from each of the combined urines. All components were analyzed for their N\(^{15}\) content. Attention is called to the fact that in no instance was a carrier employed in the isolation procedure; consequently, the isotopic values
to be reported later represent those which actually existed in the compounds at the time the subjects were killed.

For the isolation of amino acids, 30 and 40 gm. of the carcass materials derived from the animals of Groups A and C, respectively, were hydrolyzed by refluxing for 23 hours with 300 ml. of 6 N hydrochloric acid. Each hydrolysate was concentrated in vacuo to a syrup and freed from most of the excess hydrochloric acid by repeated addition of water and evaporation under reduced pressure. The final products were dissolved in water and filtered. The combined filtrate and washings from each preparation amounted to approximately 1000 ml. Each was adjusted in reaction to pH 3 and treated with charcoal (Darco G-60) for the removal of tyrosine and phenylalanine, as described by Partridge (14). The tyrosine was separated from the phenylalanine by means of its insolubility in cold water. Solution of the crude tyrosine in hot water, followed by decolorization, yielded the pure amino acid, as indicated by the accompanying analytical data. No attempt was made to recover the phenylalanine.

(2) C$_4$H$_9$O$_2$N. Calculated. C 59.66, H 6.12, N 7.73

Found (Group A). " 59.72, " 6.21, " 7.84

" ( " C). " 60.09, " 6.08, " 7.78

Cystine was isolated from the filtrates remaining after the removal of the aromatic amino acids. For this purpose, each solution was concentrated in vacuo to approximately 200 ml., adjusted to pH 5.5, and allowed to stand for several days in the cold room. The crude cystine so obtained was repeatedly crystallized until pure by dissolving in hydrochloric acid and adding solid sodium acetate until a negative test for mineral acid was given by Congo red paper.

(3) C$_6$H$_4$O$_2$N$_2$S$_2$. Calculated. C 29.99, H 5.03, N 11.66

Found (Group A). " 30.11, " 4.99, " 11.69

" ( " C). " 29.75, " 5.09, " 11.53

For the recovery of aspartic and glutamic acids, each of the solutions from which the cystine had been crystallized was diluted to 1 liter and stirred repeatedly with Amberlite IRA-400 in the hydroxyl phase. During this process, the pH was progressively raised from 5.5 to approximately 7.7. The dicarboxylic amino acids were eluted from the resin with 4 per cent hydrochloric acid, glutamic acid being recovered as the hydrochloride and aspartic acid as the copper salt. Free glutamic acid was obtained by dissolving the hydrochloride in water, adjusting the pH to 3.5 by the dropwise addition of pyridine, and treating the resulting solution with 3 to 4 volumes of ethanol. On standing overnight in the cold, glutamic acid was deposited and was removed by filtration. Purification was accomplished by reconversion into the hydrochloride, liberation of the free amino acid
as before, and crystallization from water and ethanol. Analyses of the final products are shown below.

(4) CaHgOdN. Calculated. C 40.81, H 6.17, N 9.52
Found (Group A). " 40.57, " 5.94, " 9.43
" ( " C). " 41.02, " 6.14, " 9.41

The copper aspartate was dissolved in 1 N hydrochloric acid and decomposed with hydrogen sulfide. The filtrate and washings from the copper sulfide were concentrated in vacuo to a syrup and dissolved in 10 ml. of warm water. The pH of the solution, which at this point was approximately 1.0, was raised to 3.5 by the addition of pyridine and the whole was treated with 3 to 4 volumes of ethanol. After standing overnight in the cold, the white crystals of aspartic acid were removed by filtration and recrystallized from aqueous ethanol until analytically pure.

(5) CaHgOdN. Calculated. C 36.09, H 5.30, N 10.52
Found (Group A). " 36.14, " 5.27, " 10.46
" ( " C). " 36.13, " 5.32, " 10.69

The four amino acids, the isolation of which has been outlined above, obviously are non-essential components of the food of the rat (15). For comparative purposes, it seemed desirable to obtain from the tissues an amino acid which, for the species in question, is a typical member of the group of indispensable dietary constituents. Histidine was chosen for this purpose. It was isolated from the hydrolysates remaining after the adsorption of glutamic and aspartic acids. The pH of these solutions had been raised to 7.7 incidental to the removal of the dicarboxylic amino acids. Each solution was treated as follows: The pH was first reduced to 5.5 by the cautious addition of hydrochloric acid, and the resulting fluid was slowly percolated through a column of Amberlite IRC-50 which previously had been buffered to pH 4.7 with an acetate-acetic acid mixture. After all of the hydrolysate had passed through the column, the effluent and washings were set aside for the isolation of proline.

The basic amino acids were then eluted from the resin by percolating a 4 per cent hydrochloric acid solution through the column until the eluate gave a negative ninhydrin test. The excess of hydrochloric acid was removed by concentration to dryness in vacuo three times, the residue being taken up successively in 500 ml. portions of distilled water. The final solid was dissolved in 600 ml. of water, and the whole was stirred with Amberlite IR-45, in the hydroxyl phase, until the pH had been raised to 6.6. The solution of basic amino acids was now passed through a second column of Amberlite IRC-50 which had been buffered to pH 7.0. At the latter pH, the resin adsorbs the lysine and arginine but permits the histidine to pass through the column. The effluent was then concentrated to a small
volume, filtered, and treated with 3,4-dichlorobenzenesulfonic acid under the conditions described by Vickery (16), and with a reagent prepared in accordance with his directions (17). After standing at a low temperature for several days, the crystalline histidine disulfonate was removed by filtration and converted into the monohydrochloride monohydrate. The latter salt was recrystallized until pure.

For the isolation of proline, the method of Bergmann (18) was employed. Unfortunately, it was successful only in the case of the protein hydrolysate obtained from the rats of Group A. An attempt was made to procure a comparable sample from the animals of Group C, but this met with failure. On other occasions in this laboratory, difficulty has been encountered with the Bergmann procedure. No entirely trustworthy method for the isolation of proline from small quantities of proteins appears to be available.

In the successful experiment, the effluent and washings from the first column of Amberlite IRC-50 were concentrated under reduced pressure to a small volume and treated with sufficient 6 N hydrochloric acid to render the solution approximately 0.25 N. An excess of ammonium rhodanilate in methanol was then added, and the resulting mixture was placed in the cold room for 2 days. The L-proline rhodanilate which separated was recrystallized from acidified methanol. To obtain the free amino acid, advantage was taken of the relative insolubility of pyridine rhodanilate in water, as suggested by Bergmann. Accordingly, the proline rhodanilate was suspended in 50 ml. of distilled water containing 3 ml. of reagent grade pyridine and agitated intermittently for several hours. After filtering the solution and washing the precipitate with cold water, a pink fluid was obtained. The chromogenic material was not removed by charcoal; therefore, the solution was taken to dryness by lyophilization, dissolved in water, and lyophilized again. The solid so obtained was repeatedly crystallized by dissolving it in absolute ethanol, filtering, and adding anhydrous ether. All but a trace of the color which remained was removed by extracting the solid with a small portion of ice-cold, absolute ethanol. Further crystallization of this product yielded analytically pure proline.

All of the isolated amino acids described above were tested for homogeneity by paper chromatography. Each exhibited a single, discrete spot and an \( R_f \) value which was identical with that of a pure sample of the amino acid when the isolated compound and its control were chromatographed simultaneously on the same strip of filter paper.
In addition to the tissue amino acids, a sample of urea was isolated as its dixanthydryl derivative from each of the mixed urines collected from the animals of Groups A and C. For this purpose, an appropriate aliquot of urine was decolorized with charcoal, acidified strongly with glacial acetic acid, and treated with a reagent consisting of xanthydrol dissolved in a mixture of absolute methanol and glacial acetic acid. When precipitation was complete, the dixanthydryl urea was removed by filtration through a sintered glass funnel, washed with 66 per cent acetic acid, and purified by crystallization from a dioxane-water mixture.

\[ \text{C}_{27}\text{H}_{29}\text{O}_{2}\text{N}_4 \]

Calculated. \[ N \] 6.66

Found (Group A). \[ N \] 6.57

\[ ( \text{" Group C). " } 6.64 \]

In analyzing the compounds isolated from the tissues and urines, carbon and hydrogen were determined in the Microanalytical Laboratory of this Department. Total nitrogen was estimated by the micro-Kjeldahl procedure, a mixed catalyst composed of potassium sulfate, copper sulfate, and selenium dioxide being employed, and use being made of a prolonged digestion period to insure complete oxidation (cf. Chibnall et al. (19)). In measuring the isotopic nitrogen content, each sample was first digested as in the total nitrogen determinations, after which the resulting ammonia was converted into gaseous nitrogen by the action of an alkaline hypobromite solution. The latter step was carried out in a small reaction vessel to which was attached a tipping flask containing the hypobromite. Both containers were connected to a high vacuum system. After the system had been evacuated to \( 10^{-5} \) mm. of mercury, the hypobromite was tipped into the ammonia solution and heat was applied gently with a microburner. As the nitrogen gas was liberated, it passed through a U trap which was immersed in a liquid nitrogen bath to remove water vapor and any other condensable gases. As soon as the evolution of nitrogen had ceased, the gas was transferred to a spectrometer bulb, which was then disconnected from the vacuum line and attached to the mass spectrometer. The \( \text{N}^{15} \) analyses were performed with a Consolidated-Nier isotope ratio mass spectrometer, model No. 21-201. As a check against the possibility of contamination by air, all samples were routinely tested for the presence of molecular oxygen of mass 32. Contamination so indicated was well within the 3 per cent allowed by previous workers (20), falling as a rule between 0.5 and 1.0 per cent.

**Results**

The findings in the two groups of animals are summarized in Tables II and III. In Table II is presented a partial \( \text{N}^{15} \) balance for each dietary regime. Since the carcass proteins and their component amino acids were
of primary interest for the purposes of this investigation, no attempt was made to recover all of the administered isotope. In comparable experiments involving the administration of $^{15}$N-labeled amino acids, others have shown that the non-protein nitrogenous constituents of the tissues may contain 7.8 to 11.7 per cent of the isotope (21, 22). The $^{15}$N content of these components was not determined in our tests. Furthermore, since the subjects were sacrificed immediately after they had consumed the last of the allotted food, some loss of isotopic urea must have occurred when the alimentary tracts were washed out. The lower percentage recovery of $^{15}$N in the animals of Group A, as contrasted with those of Group C, may have been due to the smaller sample of carcass protein which was available for analysis, since the rats receiving the amino acid diet gained less than did their litter mates upon the casein ration.

The data in Table II demonstrate clearly that the degree of tissue incorporation of urea nitrogen is much greater when the basal diet carries the essential amino acids only (Group A) than when all amino acids are furnished in the form of casein (Group C). Of the administered isotope, that found in the tissue proteins amounted to 21.66 and 3.05 per cent, respectively. In contrast to these findings, twice as much $^{15}$N was excreted in the urines by the animals of Group C as by those of Group A. These results were to be expected in view of the earlier observations of Rose et al. (7) and of Bloch (6), to which reference has already been made. The figures confirm the conclusion that the nitrogen of urea can be utilized

**Table II**

*Partial Balance of $^{15}$N after Feeding Isotopic Urea*

Each group, composed of three weanling rats, consumed 2.165 gm. of isotopic urea containing 158.8 mg. of excess $^{15}$N.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th></th>
<th>Group C</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Total $^N$</td>
<td>$^{15}$N</td>
<td>Total $^N$</td>
<td>$^{15}$N</td>
</tr>
<tr>
<td></td>
<td>content</td>
<td>content per cent</td>
<td>recovered</td>
<td>content per cent</td>
</tr>
<tr>
<td></td>
<td>gm.</td>
<td>mg. per cent</td>
<td>mg.</td>
<td>mg. per cent</td>
</tr>
<tr>
<td>Carcass protein</td>
<td>5.20</td>
<td>0.618</td>
<td>34.40</td>
<td>21.66</td>
</tr>
<tr>
<td>Urine</td>
<td>0.83</td>
<td>7.251</td>
<td>64.12</td>
<td>40.38</td>
</tr>
<tr>
<td>Feces</td>
<td>0.45</td>
<td>4.776</td>
<td>22.94</td>
<td>14.44</td>
</tr>
<tr>
<td>Total $^{15}$N</td>
<td>121.46</td>
<td>76.48</td>
<td>136.77</td>
<td>86.11</td>
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</table>

The data in Table II demonstrate clearly that the degree of tissue incorporation of urea nitrogen is much greater when the basal diet carries the essential amino acids only (Group A) than when all amino acids are furnished in the form of casein (Group C). Of the administered isotope, that found in the tissue proteins amounted to 21.66 and 3.05 per cent, respectively. In contrast to these findings, twice as much $^{15}$N was excreted in the urines by the animals of Group C as by those of Group A. These results were to be expected in view of the earlier observations of Rose et al. (7) and of Bloch (6), to which reference has already been made. The figures confirm the conclusion that the nitrogen of urea can be utilized.
when the subjects are in need of this element for synthetic purposes. No entirely satisfactory explanation can be offered to account for the much higher fecal output of the isotope by the members of Group A. Possibly, the explanation is to be found in the mild laxative action of diets containing mixtures of amino acids, thereby leading to more rapid passage of food through the alimentary tract and less complete absorption of the urea. Perhaps a more likely explanation is one suggested by Ratner et al. (22) to account for the isotopic content of the feces following the administration of labeled glycine. These investigators believe that the isotope "entered the intestinal lumen with the proteins of the intestinal secreta, which, like the proteins and the other nitrogenous constituents of the animals, must have contained N\textsuperscript{15}.”

One would anticipate that, under the conditions of our experiments, the

<table>
<thead>
<tr>
<th>Table III</th>
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<tbody>
<tr>
<td>N\textsuperscript{15} Content of Isolated Products</td>
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<tr>
<td>Table:</td>
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<td>----------</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Cystine</td>
</tr>
<tr>
<td>Glutamic acid</td>
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<tr>
<td>Aspartic acid</td>
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<tr>
<td>Proline</td>
</tr>
<tr>
<td>Histidine-HCl-H\textsubscript{2}O</td>
</tr>
<tr>
<td>Urea (urinary)</td>
</tr>
</tbody>
</table>

rats of Group A would need nitrogen primarily for the synthesis of the non-essential amino acids, and that a comparable need would not be experienced by the subjects of Group C. If this assumption is correct, the non-essential amino acids derived from the animals of Group A should be labeled more highly than the corresponding amino acids obtained from the members of Group C. This proved to be the case to an astonishing degree, as exemplified by the data in Table III. Omitting for the moment the values for tyrosine, one will observe that the figures representing the N\textsuperscript{15} content of the non-essential amino acids of Group A are quite high; indeed, they are 14 to 16 times the values given for the corresponding compounds obtained from Group C. The highest isotopic concentrations were observed in glutamic and aspartic acids. This is in keeping with the well known role of these amino acids in transamination reactions (cf. (23)).

With respect to tyrosine, the high isotopic content of the sample from Group A was unexpected. An abundance of evidence is available which demonstrates that tyrosine has its origin in phenylalanine (24, 25), and that
the conversion of the latter into the former occurs despite the presence or absence of preformed tyrosine in the food (25). In the present study, the ration of the animals of Group A was devoid of tyrosine; therefore, all of the tyrosine utilized by the organism for growth purposes must have been formed from phenylalanine. Little is known concerning the mechanism involved in this reaction, although an enzyme system has been described which is capable of catalyzing the over-all oxidation (26). The fact that the tyrosine was so highly labeled with N\textsuperscript{15} constitutes strong presumptive evidence against a direct conversion of the benzene ring of phenylalanine into the phenol ring of tyrosine. In the light of our data, it appears much more reasonable to assume that deamination precedes oxidation, and that a phenolic intermediate undergoes reamination with the formation of tyrosine.

In contrast to the isotopic levels observed in the non-essential amino acids, the values for the two samples of histidine (Table III) were much lower. Since this amino acid is an indispensable dietary component for the rat (15), the only mechanism whereby N\textsuperscript{15} could have entered the molecule is by a "continuous process of successive deamination and amination," as observed by Schoenheimer et al. (27) following the administration of isotopically labeled ammonium citrate. The latter compound was added to a diet containing 16 per cent of casein. The histidine isolated from the carcass protein was shown to have an N\textsuperscript{15} content of 0.013 atom per cent excess, all of which was located in the \(\alpha\)-amino group. This value is comparable to that observed in the present investigation in the animals of Group C, which also received a normal casein ration. On the other hand, the isotopic content of the histidine obtained from the rats of Group A, though decidedly lower than the levels present in the non-essential amino acids, was several times greater than that of the histidine derived from Group C. Possibly, this latter fact may be accounted for by an intensification of the process of deamination and amination incidental to the simultaneous biosynthesis of all of the non-essential amino acids. Indeed, a shift of nitrogen from one amino acid to another, or from and to the nitrogen "pool," must occur with remarkable facility inasmuch as Diet A (Table I), even without an additional source of nitrogen, permits slow growth (cf. (7)). Since Diet A contained only the essential amino acids, and these at their minimal levels, gains in weight would have been impossible were it not for the use of some of the nitrogen of the essential acids in the biosynthesis of the non-essential acids. This process should have afforded sufficient opportunity for the histidine derived from the animals of Group A to have become more heavily labeled than the histidine origi-
nating in the rats of Group C, which were not confronted with a deficient supply of nitrogen.

With respect to the urea samples isolated from the urines of the two groups of animals (Table III), little need be said. Obviously, the isotopic urea which was not used by the animals of Group A experienced very little dilution with non-isotopic urea before being excreted. The urinary sample contained 14.046 atom per cent excess of N\textsuperscript{15}, while the administered urea contained, it will be recalled, 14.75 atom per cent excess. Not more than a small decrease in isotopic content was to have been expected, since the physiologically active amino acids of Diet A furnished only 0.81 per cent of total nitrogen. On the other hand, the sample of urea derived from the rats of Group C was much lower in its isotopic content (4.884 atom per cent excess). Evidently, it had been diluted by the urea arising in the organism during the metabolism of the 18 per cent casein ration.

Finally, attention is called to the fact that the experiments described above offer no explanation of the mechanism whereby the nitrogen of urea is rendered available for the uses of the organism. Recent studies of this aspect of the problem indicate that in the mouse (28) and in the rat (3, 29) the urease activity of gastrointestinal microorganisms is largely or entirely responsible for the decomposition of urea. In the hope that the growth technique might throw more light on this point, Diet A was supplemented with urea and administered to two groups of rats, one group of which also received the mixture of antibiotic agents used by Dintzis and Hastings (28) in studying the short time fate of urea labeled with C\textsuperscript{14}. At frequent intervals, fecal samples were obtained from our animals and subjected to bacterial counts.\textsuperscript{4} Unfortunately, convincing growth data could not be obtained. The bacterial counts dropped promptly, as reported by Dintzis and Hastings, but rose again within a few days as the microorganisms acquired resistance to the drugs. This eventuality defeated the purpose of the experiments and rendered the findings inconclusive.

**SUMMARY**

Urea labeled with N\textsuperscript{15} has been administered to two groups of growing rats which were maintained on diets containing, respectively, (a) a mixture of the essential amino acids at their minimal levels (Group A), and (b) a normal ration containing 18 per cent of casein (Group C). The distribution of the isotope in the excreta and carcass proteins indicated an ex-

\textsuperscript{4} We are deeply indebted to Dr. A. B. Hastings and Dr. R. Z. Dintzis for giving us full information concerning the composition of the media used by them in making the bacterial counts, and for other useful information. We are also grateful to Dr. I. C. Gunsalus for helpful advice regarding the bacteriological tests and to Mr. John R. Stamer of the Department of Bacteriology, University of Illinois, who actually made the counts.
tensive utilization of the urea nitrogen by the subjects of Group A, but not by those of Group C. Cystine, glutamic acid, and aspartic acid isolated from the animals of the first group contained the isotope in high concentrations. On the other hand, histidine, an essential amino acid for the rat, had a low level of N$^{15}$. The data are believed to provide unequivocal proof that the nitrogen of urea can be utilized for the synthesis of the non-essential amino acids when the latter are excluded from the food, and when no other source of nitrogen is available for the purposes in question.

Tyrosine isolated from the animals of Group A, like the other non-essential amino acids, was found to contain a high level of N$^{15}$. This amino acid, under the conditions of the experiment, must have originated in the phenylalanine of the ration. The high isotopic content is believed to provide strong presumptive evidence against a direct oxidation in the para position and to suggest that deamination of the amino acid must precede the conversion of the benzene group into the phenolic group.

The probable role of bacteria in the utilization of urea nitrogen is discussed.

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UREA AS A SOURCE OF NITROGEN FOR THE BIOSYNTHESIS OF AMINO ACIDS
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