THE BIOLOGICAL FORMATION OF SARCOSINE*

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Methylglycine was first prepared in 1847 by Liebig (1) as a product of the degradation of creatine by a concentrated solution of barium hydroxide. Liebig named the new compound sarcosine. Despite its early preparation, it appears that the occurrence of sarcosine in higher animals has never been demonstrated. Indeed the only reference we have been able to find on the occurrence of sarcosine in nature is the report of Kossel and Edlbacher (2) in 1915 on the isolation of sarcosine from the radial ceca of the starfish, Astropecten aurantiacus. In the present communication evidence is presented that sarcosine is a metabolite in the rat.

The problem of sarcosine formation in this species was approached by applying the non-isotopic carrier technique to the intact animal. Sarcosinuria was produced by feeding rats a diet containing 5 per cent sarcosine. After several days, 0.1 per cent radiobetaine hydrochloride was added to the diet of one animal and 0.1 per cent radiomethionine was added to the diet of a second animal in the expectation that these radiomethyl compounds would contribute to the formation of any sarcosine that might be produced by the rat. The urine excreted during the next 24 hours was collected and sarcosine was isolated as the β-naphthalenesulfonyl derivative. The β-naphthalenesulfonylsarcosine obtained in each of the experiments was recrystallized several times and examined for the presence of C14. Both preparations were found to be radioactive.1

Evidence that the radioactivity was not due to a contaminant was provided by converting the β-naphthalenesulfonylsarcosine to sarcosine hydrochloride without a loss in specific activity. Furthermore, when the sarcosine hydrochloride was converted to creatine hydrate, the specific activity remained unchanged.2 Additional evidence that the C14 was a

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1 Similar results were obtained when 1 gm. of non-isotopic sarcosine and 4.3 mg. of radiobetaine hydrochloride were injected intraperitoneally during the course of 24 hours.

2 It was shown in control experiments that the radioactivity of the isolated sarcosine could not be attributed to contamination with radioactive creatine or radioactive creatinine.
part of the sarcosine molecule was obtained by converting the creatine hydrate to creatinine hydrochloride and thence to creatinine potassium picrate without a loss in specific activity. The specific activities of these compounds are given in Table I.

Samples of the radiosarcosine hydrochloride were degraded with silver oxide and the methylamine was isolated as the chloroplatinate. The radioactivity of the methyl carbon accounted for all of the C\textsuperscript{14} present in the radiosarcosine isolated in both the betaine and methionine experiments (Table I). These results indicate that sarcosine is formed by the rat and that the methyl groups of betaine and methionine are sources of the methyl carbon of sarcosine.

<table>
<thead>
<tr>
<th>Compound fed</th>
<th>Sarcosine derivative</th>
<th>Compound fed</th>
<th>Radiobetaine</th>
<th>Radiomethionine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.p.m. per mg</td>
<td>c.p.m. per mg</td>
</tr>
<tr>
<td>(\beta)-Naphthalenesulfonysarcosine</td>
<td>3.2 \times 10^4</td>
<td>Radiobetaine</td>
<td>5.8 \times 10^4</td>
<td></td>
</tr>
<tr>
<td>Sarcosine hydrochloride</td>
<td>3.2 \times 10^4</td>
<td>Radiomethionine</td>
<td>5.3 \times 10^4</td>
<td></td>
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<tr>
<td>Creatine hydrate</td>
<td>3.4 \times 10^4</td>
<td></td>
<td>5.2 \times 10^4</td>
<td></td>
</tr>
<tr>
<td>Creatinine chloride</td>
<td>3.2 \times 10^4</td>
<td></td>
<td>5.4 \times 10^4</td>
<td></td>
</tr>
<tr>
<td>&quot; potassium picrate</td>
<td>3.2 \times 10^4</td>
<td></td>
<td>5.8 \times 10^4</td>
<td></td>
</tr>
<tr>
<td>Methylamine chloroplatinate*</td>
<td>3.5 \times 10^4</td>
<td></td>
<td>6.3 \times 10^4</td>
<td></td>
</tr>
</tbody>
</table>

* Prepared from the radiosarcosine hydrochloride. Since the chloroplatinate contains 2 moles of methylamine per mole, its specific activity is expressed as counts per minute per 0.5 mg.

It is of interest to estimate the percentage of radiomethyl groups converted to sarcosine under the conditions employed. In both the betaine and methionine experiments, approximately 15 per cent of the ingested non-isotopic sarcosine was recovered from the urine. If it is assumed that the radiosarcosine recovered from the urine represented 15 per cent of the radiosarcosine synthesized, it may be calculated that 5.3 per cent of the methyl carbon of the ingested radiobetaine and 2.8 per cent of the methyl carbon of the ingested radiomethionine were converted to sarcosine in the body in 24 hours. Since betaine contains 3 times as many methyl groups as methionine and has a lower molecular weight than methionine, it may be calculated from these percentages, and from the food consumption, that approximately 8 times as much radiosarcosine was formed from the radiobetaine hydrochloride as from the radiomethionine. In this connection it should be noted that Dubnoff (3) has shown that homocysteine is methyl-
ated in the presence of betaine to yield methionine, and Muntz (4) has shown that dimethylglycine is a product of the reaction. If all, or most, of the radiosarcosine formed in our betaine experiment arose from the direct conversion of betaine to sarcosine by demethylation (with dimethylglycine as an intermediate), then approximately 16 per cent of the dietary betaine was converted to sarcosine. However, the conversion of significant quantities of betaine methyl carbon to sarcosine methyl carbon through other reactions cannot be excluded.

The experiments reported in this paper are of particular interest in view of parallel investigations (5) carried out in this laboratory on the metabolism of sarcosine. Direct proof of the conclusion of Handler, Bernheim, and Klein (6) that formaldehyde is produced in the oxidation of sarcosine by homogenized liver was provided by isolating formaldehyde from liver homogenates incubated with sarcosine. The origin of the formaldehyde from the methyl group was shown conclusively in experiments with radiosarcosine. Radioformaldehyde was also isolated from surviving liver slices incubated with radiosarcosine, and a trace of radioformaldehyde was found in the urine of a rat injected with radiosarcosine. Similarly, formic acid was shown to be a product of the oxidation of the methyl group of sarcosine by liver homogenates, liver slices, and the whole animal.

Still another single carbon compound has been identified as a product of sarcosine oxidation by a biological preparation. In 1944, Ratner, Nocito, and Green (7) reported that sarcosine was converted to methylamine and glyoxylic acid by a highly purified preparation of glycine oxidase obtained from the acetone-insoluble fraction of minced hog kidney. The methylamine was isolated and identified as the picrolonate.

EXPERIMENTAL

Metabolism Experiments—Two male rats of the Rockland Farms strain, weighing approximately 125 gm. each, were placed on a purified diet containing 5 per cent of non-isotopic sarcosine.3 After maintaining a constant weight for 2 days the rats began to gain at a rate of approximately 3 gm. a day. No toxic symptoms were observed during the course of the experiments.

On the 6th day, 0.1 per cent of betaine hydrochloride labeled with C14 in the methyl groups (9) was added to the diet of one animal and 0.1 per cent of L-methionine labeled with C14 in the methyl group (10) was added to the diet of the second animal. The urine excreted during the next 24 hours was collected in 1 ml. of 1 N hydrochloric acid. During the collection

3 The percentage composition of the basal diet was as follows: sucrose 50.4, casein 20, Covo 19, corn oil 1, L-cystine 0.4, betaine 0.2, sarcosine 5, Salts 4 (8), and vitamins (8).
period the rat fed radiobetaine ate 11.6 gm. of diet and the one given radiomethionine ate 8.4 gm. of diet.

Isolation of \( \beta \)-Naphthalenesulfonylsarcosine. Sarcosine was isolated from urine as the \( \beta \)-naphthalenesulfonyl derivative by a modification of the procedure described by Friedmann (11). The urine was adjusted to pH 8 to 9 with sodium hydroxide and filtered. 750 mg. of \( \beta \)-naphthalenesulfonylchloride in 25 ml. of ethyl ether were added and the mixture was stirred vigorously for 3 hours. The pH was maintained at 8 to 9 during the course of the reaction by the addition of sodium hydroxide when necessary. At the end of 3 hours, the ether phase was removed and the aqueous phase was extracted twice with 35 ml. portions of ether.

The aqueous phase was filtered, acidified to Congo red violet hydrochloric acid, and placed in the refrigerator overnight. The \( \beta \)-naphthalenesulfonyl-sarcosine was removed by filtration and recrystallized twice from alkaline solution by the slow addition of hydrochloric acid, and twice from hot 22 per cent ethanol. The capillary melting point of the \( \beta \)-naphthalenesulfonyl-sarcosine isolated in both experiments was 171–172°, corrected. 280 mg. of the derivative were isolated from the urine of the rat which was fed radiobetaine and 178 mg. from the urine of the rat given radiomethionine. These quantities are equivalent to 15.2 per cent and 13.4 per cent, respectively, of the carrier sarcosine ingested during the 24 hour collection period.

52.3 mg. of the \( \beta \)-naphthalenesulfonylsarcosine isolated in the radiobetaine experiment were transferred to a filter paper disk and counted by the procedures described in an earlier publication (12). The crystals gave 1008 c.p.m. above background, or 6038 c.p.m. when corrected for self-absorption. The activity of the total amount of \( \beta \)-naphthalenesulfonyl-sarcosine isolated from the urine was 5.36 times this figure, or 3.24 \( \times \) 10^4 c.p.m., corrected. This was equivalent to the activity of 93 \( \gamma \) of the ingested radiobetaine hydrochloride. If it is assumed as a first approximation that the radiosarcosine recovered from the urine represented 15.2 per cent of the quantity formed by the rat (15.2 per cent of the ingested carrier sarcosine was excreted), then 612 \( \gamma \) of the 11.6 mg. of ingested radiobetaine hydrochloride, or 5.3 per cent, were converted to sarcosine during the 24 hours of the experiment. On the basis of methyl groups, 1 molecule of betaine is equivalent to 3 molecules of sarcosine. In these experiments all of the C\(^{14}\) of the isolated sarcosine was located in the methyl group, as is shown below. Therefore, approximately 1.1 mg. of methyl-labeled sarcosine were formed from the radiobetaine in 24 hours. Similarly, 2.8 mg. of non-isotopic sarcosine were formed from the non-isotopic betaine consumed in the diet during the same period.

In the radiomethionine experiment, 47.4 mg. of the \( \beta \)-naphthalene-
sulfonylsarcosine isolated from the urine gave 182 c.p.m. above background, or 992 c.p.m. when corrected for self-absorption. The corrected value for the total amount of sarcosine derivative isolated was 3.75 times this figure, or $3.72 \times 10^9$ c.p.m. This was equivalent to the activity of 31 $\gamma$ of the ingested radiomethionine. On the basis of the 13.4 per cent of carrier sarcosine isolated from the urine, it was calculated that 231 $\gamma$ of the 8.4 mg. of ingested radiomethionine, or 2.8 per cent, were converted to sarcosine. Thus 0.14 mg. of methyl-labeled sarcosine was formed from the ingested radiomethionine in 24 hours. If the metabolism of the methionine of the dietary casein followed the metabolism of the radiomethionine, then an additional 1 mg. of sarcosine was synthesized from \textit{exogenous} non-isotopic methionine during this same period, since casein contains approximately 3.5 per cent methionine (13).

The number of counts per minute per mm of the $\beta$-naphthalenesulfonylsarcosine isolated in the betaine and methionine experiments is given in Table I.

\textit{Sarcosine Hydrochloride}—100 to 300 mg. of $\beta$-naphthalenesulfonylsarcosine were suspended in 2 ml. of concentrated hydrochloric acid and heated in a sealed tube at 100° for 22 hours (14). The $\beta$-naphthalenesulfonic acid crystals were removed by filtration and washed with a small amount of ice-cold hydrochloric acid. The hydrochloric acid was removed from the filtrate at the water pump and the residue was dissolved in warm absolute alcohol. The sarcosine hydrochloride was crystallized four times from alcoholic solution by the slow addition of ether. It was then de-colorized with Darco and finally recrystallized from hot glacial acetic acid.

Prior to hydrolysis, the radioactive $\beta$-naphthalenesulfonylsarcosine isolated in the radiobetaine experiment was diluted 3-fold with non-isotopic $\beta$-naphthalenesulfonylsarcosine. The material isolated in the radiomethionine experiment was converted directly to sarcosine hydrochloride, recrystallized, and diluted 3-fold with non-isotopic sarcosine hydrochloride. In both experiments the capillary melting point of the radiosarcosine hydrochloride was 170°, corrected. For convenience of comparison, the specific activity of these and subsequent preparations has been calculated on the basis of the undiluted material (Table I).

\textit{Creatine and Creatinine}—25 mg. of radiosarcosine hydrochloride were treated with cyanamide under the conditions described in an earlier publication (15). The crystals of creatine hydrate were centrifuged, and washed several times with a few drops of ice-cold water. The creatine hydrate was recrystallized from water by the addition of alcohol and its radioactivity measured in the usual manner.

The creatine hydrate was converted to creatinine hydrochloride by refluxing in 2 ml. of 1 N hydrochloric acid for 3 hours. The creatinine
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Hydrochloride was recrystallized from water and acetone and its specific activity determined.

Creatinine potassium picrate was prepared from the creatinine hydrochloride, and the potassium picrate was recrystallized once from 0.2 per cent picric acid half neutralized with potassium hydroxide, and once from water. The creatinine potassium picrate obtained in both experiments was pure according to analysis by the Jaffé reaction. The specific activities of the compounds described above are given in Table I.

**Methylamine Chloroplatinate**—20 mg. samples of the radiosarcosine hydrochloride prepared in each of the two experiments were degraded with silver oxide according to the method of Herbst and Clarke (16). The methylamine formed was aerated into 1 N hydrochloric acid. The acid was removed *in vacuo*, the residue was dissolved in 4 to 5 ml. of absolute alcohol, and methylamine chloroplatinate was precipitated by the dropwise addition of a saturated alcoholic solution of platinum tetrachloride. The methylamine chloroplatinate, following three recrystallizations from water and alcohol, melted on the micro block at 223–224°, corrected. The platinum content of both preparations agreed with the calculated value.

In each experiment the radioactivity of the methylamine chloroplatinate accounted for the C\(^{14}\) content of the isolated sarcosine. Since the chloroplatinate contains two methyl groups per mole, the specific activity was calculated as counts per minute per 0.5 mm (Table 1).

**Control Experiments**—In order to justify the conversion of the isolated radiosarcosine to radiocreatinine as a test of the purity of the former compound, it was necessary to show that any radiocreatinine or radiocreatine that might have been excreted in the urine of the experimental animals was not carried along as a contaminant in the isolation of sarcosine. The following experiments were therefore carried out. 100 mg. of non-isotopic sarcosine and either 10.2 mg. of radiocreatinine\(^{4}\) (8.5 \times 10^4 c.p.m., corrected) or 10 mg. of radiocreatine\(^{4}\) (7.3 \times 10^4 c.p.m., corrected) were dissolved in 1 ml. of 1 N hydrochloric acid in the collection flask of a metabolism apparatus. The urine excreted by a rat in the next 24 hours was collected and sarcosine was isolated as the \(\beta\)-naphthalenesulfonyl derivative. In both instances the recrystallized derivative, when counted in pads of “infinite” thickness, gave less than 1 c.p.m. above the background.

\(^{4}\) Diluted samples of the radiocreatinine and radiocreatine described in a previous publication (15).
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pad of “infinite” thickness. This activity was negligible compared with the activity of the β-naphthalenesulfonylsarcosine isolated from the urine of the rat fed radiobetaine. Moreover, when the material isolated in the control experiment was converted to sarcosine hydrochloride, the counts dropped to less than 1 c.p.m. above the background.

In the radiomethionine experiment, the β-naphthalenesulfonylsarcosine, when counted in a pad of “infinite” thickness, gave 460 c.p.m. above background. However, when this material was converted to sarcosine hydrochloride, the counts dropped to 86 c.p.m. for a 34 mg. sample, and the creatine hydrate (16 mg.) derived from the sarcosine gave less than 1 c.p.m. above background. Thus, any C\textsuperscript{14} present in the urine of the experimental animals in the form of radiomethionine was eliminated in the conversion of the isolated sarcosine to creatine.

The authors wish to express their appreciation to Professor Vincent du Vigneaud for his advice and counsel during the course of this investigation. We wish to thank Mrs. Josephine T. Marshall for the platinum determinations.

**SUMMARY**

Rats were fed a diet containing non-isotopic sarcosine together with radiobetaine or radiomethionine labeled with C\textsuperscript{14} in the methyl group. Sarcosine was isolated from the urine as the β-naphthalenesulfonyl derivative and found to contain an appreciable degree of radioactivity.

Evidence that the C\textsuperscript{14} was incorporated in the sarcosine molecule was provided by converting the β-naphthalenesulfonylsarcosine through the following series of compounds without a loss in specific activity: sarcosine hydrochloride, creatine hydrate, creatinine chloride, and creatinine potassium picrate. The specific activity of the methyl group of the isolated sarcosine accounted for all of the C\textsuperscript{14} present in the molecule.

These results indicate that sarcosine is a metabolite in the rat and that “biologically labile” methyl groups are sources of the carbon of the methyl group of sarcosine.

**BIBLIOGRAPHY**

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