THE COMPARATIVE ACTION OF CHOLINE AND BETAINE IN EFFECTING THE REPLACEMENT OF METHIONINE BY HOMOCYSTINE IN THE DIET

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In a recent communication by du Vigneaud, Chandler, Moyer, and Keppel (1) it was reported that either choline or betaine would enable an animal to utilize homocystine or homocysteine in the diet in place of methionine. With these compounds, the white rat was able to grow on a diet which was free of methionine but which contained homocystine; whereas, in their absence, the animals were unable to grow. The obvious inference was drawn by these authors that, in the presence of choline or betaine, the body was able to methylate the sulfur of homocysteine to give methionine. An actual transfer of methyl groups was postulated. It was suggested that the reaction might be reversible and that methionine might be the precursor of choline in so far as furnishing the methyl groups is concerned. The possibility of methionine being involved in other methylations of the body was likewise pointed out (1). It is of interest in this connection that Borsook has recently found that in the presence of methionine an increased amount of guanidoacetic acid could be methylated by liver slices to form creatine (2).

The present communication deals with the relative potencies of choline and betaine in bringing about the utilization of homocystine. Preliminary work had given some indication that choline was more effective (1). This led to the tentative conclusion that betaine was probably effective in so far as it was converted to choline within the body. A more careful comparison of the effects of the two compounds was desirable. In order to rule out as far as possible the chance of bacterial conversion, it was decided...
to compare the compounds, not only by administration per os, but also by parenteral administration.

**EXPERIMENTAL**

Young white rats were placed on a diet free of cystine and methionine in which the amino nitrogen was supplied by pure amino acids in a mixture modeled after that used by Rose and Rice (3). The diet had the following composition: amino acid mixture 23.6 (1), dl-homocystine 1.25, dextrin 24.15, sucrose 15.0, salt mixture (Osborne and Mendel (4)) 4.0, agar 2.0, and corn oil (Mazola) 30.0 parts. Irradiated ergosterol, dl-a-tocopherol, and halibut liver oil (S. B. Penick and Company) were added to the corn oil in such amounts as to provide 20 U.S.P. units of vitamin D activity, 0.05 mg. of dl-a-tocopherol, and 200 U.S.P. units of vitamin A per rat per day. The halibut liver oil contained 90,000 U.S.P. units of vitamin A per gm., and the irradiated ergosterol in cottonseed oil 600,000 U.S.P. units per gm. The water-soluble vitamins were supplied by two pills daily, each having the following composition: thiamine chloride 10 micrograms, riboflavin 10 micrograms, nicotinic acid 10 micrograms, modified ryzamin-B (freed of choline by precipitation as the reineckate (1)) 12.5 mg., and dextrin 150 mg. With Litters I and III the modified ryzamin-B was omitted and 5 micrograms of vitamin B₆ were added to each pill. We had previously found that rats grew normally during the short periods used in these experiments when the ryzamin-B was omitted from the diet.

Rats placed on this basal diet containing homocystine failed to grow. Excellent growth resulted, however, when the proper amounts of either choline or betaine were administered. In order to compare the relative effectiveness of these two compounds in producing growth they were given at suboptimal or minimal levels. The method of administration was either in the pill with the water-soluble vitamins, or by subcutaneous injection twice daily.

1 The authors wish to thank Dr. J. Waddell of E. I. du Pont de Nemours and Company, Inc., for the irradiated ergosterol, Dr. R. D. Shaner of Hoffmann-La Roche, Inc., for dl-a-tocopherol (ephynal), Dr. D. F. Robertson of Merck and Company, Inc., for thiamine chloride (betabion), riboflavin, and vitamin B₆ hydrochloride, and Mr. W. O. Frohring of General Biochemicals, Inc., for nicotinic acid.
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Litter I was placed on the diet described above but without the modified ryzamin-B at approximately 3 weeks of age. After a 4 day preliminary period of adjustment to the diet and the experimental cages, two rats were given orally 10 and 25 mg. of choline chloride per day, respectively, two others were given equivalent amounts of betaine chloride, and one was allowed to continue without either supplement. The two rats receiving choline began to grow immediately and continued to gain weight (approximately 1 gm. per day) during the 16 days on these supplements. The control animal, and the two rats receiving betaine, all died within 7 days.

Another litter of rats, Litter II, was started at approximately 3 weeks of age and betaine was given at high levels. These animals were given modified ryzamin-B. Two rats were given orally 10 and 25 mg. of choline chloride per day, respectively, two were given equivalent amounts of betaine chloride, and two others were given 55 mg. of betaine chloride. The growth curves of

Fig. 1. Growth curves of Litter II showing the effect of various levels of betaine and choline, which are given in mg., administered *per os*.
Choline and Betaine

this litter are shown in Fig. 1 and the food consumption is given in Table I. All of the rats survived and gained weight except the one getting the smallest amount of betaine. The other rats receiving betaine, even at the 55 mg. level, showed poor growth compared with the choline-fed animals, particularly during the first 8 days of the experiment. Following this initial period, however, these rats showed a decided increase in the rate of growth and maintained levels only slightly inferior to those of the choline-

![Table I](image)

<table>
<thead>
<tr>
<th>Litter No.</th>
<th>Rat No. and sex</th>
<th>Days</th>
<th>Daily supplement</th>
<th>Average daily food consumption gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>167 ♂</td>
<td>1-20</td>
<td>55 mg. betaine chloride</td>
<td>3.2</td>
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<tr>
<td></td>
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<td>3.5</td>
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<tr>
<td></td>
<td>169 ♂</td>
<td>1-4*</td>
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<td>2.5</td>
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<tr>
<td></td>
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<td>1-20</td>
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<td>3.8</td>
</tr>
<tr>
<td></td>
<td>171 ♂</td>
<td>1-20</td>
<td>25 &quot; choline &quot; &quot;</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>174 ♂</td>
<td>1-20</td>
<td>10 &quot; &quot; &quot; &quot;</td>
<td>6.0</td>
</tr>
<tr>
<td>III</td>
<td>115 ♂</td>
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<td></td>
<td>116 ♀</td>
<td>1-23</td>
<td>5 &quot; &quot; &quot; &quot;</td>
<td>3.9</td>
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<td></td>
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<td>23-40</td>
<td>50 &quot; &quot; &quot; &quot;</td>
<td>4.1</td>
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<tr>
<td></td>
<td>118 ♀</td>
<td>1-40</td>
<td>No supplement</td>
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<tr>
<td></td>
<td>120 ♂</td>
<td>1-40</td>
<td>27.5 mg. betaine chloride</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>121 ♀</td>
<td>1-23</td>
<td>5.5 &quot; &quot; &quot; &quot;</td>
<td>5.0</td>
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<tr>
<td></td>
<td>122 ♀</td>
<td>23-32</td>
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<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-40</td>
<td>11 &quot; &quot; &quot; &quot;</td>
<td>5.0</td>
</tr>
</tbody>
</table>

* Died.

fed animals. It may be noted, in contrast, that choline produced an immediate and sustained growth response.

The rats of Litter III were placed on the diet free of cystine and methionine with no supplement of modified ryzamin-B at approximately 5 weeks of age. Choline was given at 5, 10, and 25 mg. levels, and betaine to three other rats in equivalent amounts. These supplements were administered by subcutaneous injection twice daily. The growth curves for this litter are given in Fig. 2 and the food consumption is shown in Table I. The results show that choline was ineffective at the level of 5 mg. daily,
but produced moderate growth at the 10 mg. level, and very good growth at the 25 mg. level. Betaine was less effective at corresponding levels. It is to be noted that the principal difference between the effect of 25 mg. of choline chloride given to Rat 115

![Diagram](image)

Fig. 2. Growth curves of Litter III showing the effect of daily subcutaneous injections of various levels (given in mg.) of choline and betaine.

and the effect of the equivalent amount of betaine given to Rat 120 was the delay of several days before growth began in the latter case.

**DISCUSSION**

The observations recorded in this paper demonstrate that betaine is less effective than choline in supporting growth of rats on a diet containing homocystine but free of cystine and
methionine. The difference is manifested principally in a delay of several days in the response of growth following the administration of betaine to deficient animals. When the rats are only 3 to 4 weeks old, this delay in action often results fatally. This is of interest in connection with the observations of Griffith and Wade (5) on the high mortality of young rats on a low choline diet. The mechanism for the effect of betaine in bringing about the transformation of homocystine to methionine appears to be absent or at least inefficient at first, but is fully developed during a period of several days after the compound is provided to the animals. This is true even when the compounds are injected.

One explanation of these results might be that choline is the compound which is actually used by the body for the methylation of homocystine but that betaine can be used after the organism acquires the ability to convert sufficient quantities of it to choline. Support for such a theory is also found in the fact that even after the first delay in action betaine is somewhat less effective than equivalent amounts of choline. This inequality of activity of the two substances could be accounted for by a less than quantitative transformation of betaine to choline.

It is realized, of course, that relative effects are only suggestive of such a conversion, and not proof, since both compounds could act directly with different inherent activities. The effect of different rates of destruction of the two compounds in the body must also be recognized as a factor in determining the amounts required to produce a given effect.

The bacterial flora are apparently not necessary agents in the use of choline and betaine for the conversion of homocystine to methionine, inasmuch as subcutaneous administration of the compounds is as effective as their inclusion in the diet. Since parenteral administration also does not alter the relative effectiveness of the two compounds, it may be justified to assume that the bacterial flora are not required for any interconversion that may occur.

**SUMMARY**

Choline has been found to be more effective than equivalent amounts of betaine in enabling an animal to utilize homocystine
in place of methionine. Both compounds are active in this respect whether administered \textit{per os} or parenterally.

The authors wish to thank Miss Doris Flavelle for her worthy assistance in this investigation.

\textbf{BIBLIOGRAPHY}


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