THE CHOLINE REQUIREMENT OF THE BABY PIG

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THIRTY FIGURES

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In a previous paper it was established that the baby pig on a 30% casein ration requires dietary choline, and the deficiency syndrome was described (Johnson and James, '48a). The object of the experiments reported in this paper was to determine the dietary choline level essential for optimum growth and for the physiological well-being of the pig.

To determine the choline requirement it is necessary to consider the methionine (or other methyl donor) content of the diet. A choline deficiency was produced in the baby pig using a diet containing approximately 0.8% methionine, based on microbiological assays of casein (Henderson and Snell, '48; Horn et al., '46; Stokes et al., '45) to 1.0% based on chemical assays (Block and Boling, '45). This suggests that in the baby pig the methylation of aminoethanol by the methyl groups from methionine does not take place to any considerable extent (compare with the chick as discussed by Jukes, '41).

EXPERIMENTAL

Two-day-old Duroc-Jersey pigs from the University farm were used in this study. The technique of feeding and care of the animals has been described (Johnson, James and Krider, '48b). The composition of the basal ration is given
in table 1. As in previous experiments, 2% sulfasuxidine was included in the ration to inhibit intestinal synthesis. Two experiments were conducted; one using ad libitum feeding to establish the range of the requirement and the second using

| TABLE 1 |
| Composition of the basal diet |
| % |
| Casein (Labco, vitamin-free) | 30.0 |
| Glucose (cerelose) | 37.4 |
| Mineral salts | 6.0 |
| Lard | 26.6 |

These materials were made up into a "milk" containing 4% lard (liquid basis) and the following amounts of vitamins per liter:

- Vitamin A 2000 I.U.
- Vitamin D$_{3}$ 200 I.U.
- Alpha-tocopherol acetate 1.0 mg
- 2-methyl-1,4-naphthoquinone 0.26 mg
- Thiamine 0.65 mg
- Riboflavin 1.33 mg
- Nicotinic acid 2.67 mg
- Pyridoxine 2.67 mg
- Ca-pantothenate 8.00 mg
- Inositol 26.00 mg
- PABA 2.67 mg
- Ca-pantothenate 0.052 mg
- Biotin 0.01 mg
- Thiamine 0.65 mg
- PABA 2.67 mg
- Riboflavin 1.33 mg
- Nicotinic acid 2.67 mg
- Pyridoxine 2.67 mg

Choline chloride was added as indicated in the text. Reticulogen was added at the rate of 0.25 ml/day in test 2.

1 Sulfasuxidine was supplied by Sharp and Dohme, Inc., Glenolden, Pa., through the courtesy of Dr. S. F. Scheidy.

2 Thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, biotin, nicotinic acid and α-tocopherol acetate were supplied by Hoffmann-La Roche, Inc., Nutley, New Jersey, through the courtesy of Dr. J. C. Bauernfeind. Pteroylglutamic acid was supplied by the Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York, through the courtesy of Dr. T. H. Jukes. Inositol was supplied by the A. E. Staley Manufacturing Co., Decatur, Illinois. Hyflavin (a highly water-soluble form of riboflavin) was supplied by Endo Products, Inc., Richmond Hill, New York.

3 Retieulogen was supplied by Eli Lilly and Co., Indianapolis, Ind.

In the first experiment 4 groups of three pigs each were fed ad libitum the basal ration plus choline chloride at the paired feeding to eliminate any effects due to differences in food intake within the limits of this range.
following levels based on the dry matter of the ration: Group I — 0.0%, II — 0.05%, III — 0.10% and IV — 0.20%. The experiment was continued for 8 weeks. At its conclusion representative pigs from each group were sacrificed and microscopic sections were made of their livers, kidneys, nerves, and skeletal muscles.

From the results of experiment 1 the dietary requirement for choline chloride appeared to be between 0.05% and 0.1%. Therefore, in the second experiment these two levels of choline were compared by pair feeding 6 pairs of pigs for 8 weeks, as in experiment 1. Reticulogen, a liver extract material, was added to the basal ration in this experiment at the rate of 0.25 ml daily to supply an essential growth factor or factors, as it had been found by the present authors in previous work that the basal diet was deficient in this respect (Neumann et al., '48). All 12 of the pigs in experiment 2 were sacrificed and microscopic sections made of their tissues as in experiment 1. In addition, ether extract determinations were made of the livers.

RESULTS

The average growth curves of the groups of pigs from both experiments are plotted in chart 1. The rates of gain of the 4 groups in experiment 1 are not significantly different, although the difference between groups I and III is indicative of a growth effect (P = 0.08). However, this effect as shown in experiment 2 and as reported previously (Johnson and James, '48a) is probably due to an increased food intake. Similar findings have been reported by Foâ, Weinstein, and Kleppel ('48) for the rat. These workers found no effect of a choline deficiency on the growth of the rat when food consumption was equalized. From the data in table 2 it is apparent that the 0.05% and 0.10% choline groups made increasingly more rapid gains on less dry matter consumed than the negative control group, although the "P" value between the gains of the 0.0% and 0.1% choline groups is only 0.08%.
Pictures of two representative pigs from each of the first three groups in experiment 1 are given in plate 1. Pigs in figures 1 and 2 are from the "0.0% choline" group; those in 3 and 4 from the "0.05% choline" group; and the animals in 5 and 6 from the "0.1% choline" group. The pigs in figures 1 to 4, and in particular 2 and 4, show the gross symptoms of choline deficiency. They are unthrifty and have poor conformation, appearing short-legged and pot-bellied. These pigs lacked coordination in their movements (as also reported by Ellis, Madsen and Miller, '43) and seemed to lack proper rigidity in the joints, particularly in the shoulders (note also pigs 8 and 9, plate 1, Johnson and James, '48a).
The microscopic sections of nerve and skeletal muscle failed to show any differences between groups.

The performance records of the baby pigs in experiment 2 are given in table 3. The two groups showed no difference in rate or economy of gain.

Three pairs of pigs from experiment 2 are pictured in plate 2. Pigs in figures 7, 9, and 11 received 0.05% choline, while those in figures 8, 10 and 12 received 0.1%. The pigs on the higher level of choline were thriftier in appearance and had better haircoats than their pair mates on the lower level.

The histological findings with respect to the livers and kidneys of pigs from both experiments are illustrated in tables 2, 3, 4 and 5. In plate 3, figures 13, 14 and 15 are photomicrographs of sections of the livers of pigs in experiment 1 receiving 0.0, 0.5 and 0.10% choline, respectively. Figures 16 and 17 are liver sections from pair-fed pigs in experiment 2 on 0.05 and 0.10% choline, respectively. Note that on the choline-free diet (fig. 13) the hepatic cells of the liver have become greatly distended with fat and the nuclei are pushed over to one side. The livers of the animals on the 0.05% level of choline contain slightly more fat globules than those from the pigs on the 0.10% level. In addition, there was a slight but significant difference in ether extract content of the livers.

### Table 2

*Response of baby pigs fed various levels of choline ad libitum for 56 days*

<table>
<thead>
<tr>
<th>DIETS FED</th>
<th>BASAL + 0.00% CHOLINE</th>
<th>BASAL + 0.05% CHOLINE</th>
<th>BASAL + 0.10% CHOLINE</th>
<th>BASAL + 0.20% CHOLINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pigs fed</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ave. initial weight (kg)</td>
<td>1.67</td>
<td>1.60</td>
<td>1.55</td>
<td>1.70</td>
</tr>
<tr>
<td>Ave. final weight (kg)</td>
<td>13.49</td>
<td>15.27</td>
<td>17.56</td>
<td>15.36</td>
</tr>
<tr>
<td>Ave. final weight / initial weight (kg)</td>
<td>8.07</td>
<td>9.53</td>
<td>11.69</td>
<td>9.22</td>
</tr>
<tr>
<td>Dry matter consumed (kg)/kg gain</td>
<td>1.22</td>
<td>1.11</td>
<td>1.07</td>
<td>1.18</td>
</tr>
</tbody>
</table>
Renal damage appeared more serious than the liver condition. The photomicrographs (plates 4 and 5) illustrate the degree of damage to the glomeruli and the tubular epithelium. On the 0.0% level all glomeruli shown are occluded and apparently non-functional (fig. 18), as contrasted with the glomeruli seen in figure 20 from a pig on the 0.1% choline level and in figure 21 from a pig on an adequate farm ration.

The photomicrographs were made of the cortical region of the kidney in all cases, as damage in this area was more pronounced. The kidneys of the pigs on the 0.05% choline level show less damage than those on the 0.0% level but are definitely abnormal. This is true in both the ad libitum feeding experiment (fig. 19) and in the paired feeding experiment (fig. 22), while the kidneys of the pigs on the 0.10% level are normal (figs. 20 and 23). The photomicrographs in plate 5 illustrate at higher magnification the kidneys on both the ad libitum and the paired feeding experiments when less than 0.10% choline was included in the diet.

**DISCUSSION**

An analysis of the data presented in this paper shows that the very young pig requires choline even in the presence of a substantial amount of methionine. In this respect the inter-

**TABLE 3**

*Effect of 0.05 and 0.10% choline in baby pig diets (equal feed intake for 56 days)*

<table>
<thead>
<tr>
<th>DIETS FED</th>
<th>BASAL DIET + 0.05% CHOLINE</th>
<th>BASAL DIET + 0.10% CHOLINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pigs fed</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Ave. initial weight (kg)</td>
<td>1.60</td>
<td>1.59</td>
</tr>
<tr>
<td>Ave. final weight (kg)</td>
<td>21.32</td>
<td>21.79</td>
</tr>
<tr>
<td>Ave. final wt. (kg)</td>
<td>13.32</td>
<td>13.07</td>
</tr>
<tr>
<td>Ave. liver weight (dry) (gm)</td>
<td>146.72</td>
<td>137.17</td>
</tr>
<tr>
<td>Ave. ether extract in liver (%)</td>
<td>5.80</td>
<td>5.20</td>
</tr>
<tr>
<td>Ave. liver ether extract/kg body wt.</td>
<td>1.34</td>
<td>1.16</td>
</tr>
<tr>
<td>Dry matter consumed (kg)/kg gain</td>
<td>1.167</td>
<td>1.140</td>
</tr>
</tbody>
</table>
relationship of choline and methionine in the baby pig is similar to that in the chick rather than that in the rat. Jukes ('40) showed that methionine will not prevent the symptom of perosis seen in choline deficiency in the chick; that is, it will not methylate aminoethanol (Jukes, '41) to form choline as it does in the rat (Du Vigneaud, Chandler, Cohn and Brown, '40). Recently McKittrick ('47) has reported that for optimum growth of 4-week-old chicks 0.5% methionine is required when optimum choline (0.55%) is included in the diet, and that 0.10% choline is required when optimum methionine (0.75%) is included in the diet. The baby pigs in our experiments received approximately the same amount of methionine (0.8%) as that which allowed optimum growth with 0.1% choline in these chick experiments. Similarly the pigs showed a definite choline requirement of the same magnitude (0.1%) as that which McKittrick reported for the chick.

However, Treadwell ('48) has recently reported that in the young (50 gm) rat the requirement for methionine on a choline-free diet is 1.3 to 1.8%. While there is no choline requirement at this level of methionine, when the diet contains only 0.8% methionine the young rat has a definite need for choline. With optimum choline the methionine requirement of the rat is 0.5–0.6% (Womack and Rose, '41).

From these data it would seem necessary to undertake further experiments involving still higher methionine levels to determine whether the baby pig can synthesize all of its required choline in the presence of adequate methionine or whether there is an absolute dietary requirement for choline.

SUMMARY

1. On a "synthetic milk" ration containing approximately 0.8% methionine (30% casein) the baby pig requires 0.1% choline in its diet.

2. It appears that in the young pig, as in the chick, the ability of methionine to supply methyl groups for the synthesis of choline from aminoethanol may be limited. The
methionine requirement of the baby pig must be established before this can be determined.

3. When the food intake is equalized choline does not have a growth stimulating effect.

4. Choline deficiency in these baby pigs resulted in gross symptoms of unthriftiness, poor conformation (short-legged and pot-bellied), lack of coordination in movements and a characteristic lack of proper rigidity in the joints, particularly the shoulders. The pigs also showed typical fatty infiltration of the livers and characteristic renal glomerular occlusion and some tubular epithelial necrosis.

LITERATURE CITED

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TREADWELL, C. R. 1948 Growth and lipotropism. II. The effects of dietary methionine, cystine, and choline in the young white rat. Ibid., 176: 1141.

WOMACK, M., AND W. C. ROSE 1941 The partial replacement of dietary methionine by cystine for purposes of growth. Ibid., 141: 375.
Figures 1 and 2 represent pigs on the 0.0% choline diet, whereas the pigs represented by figures 3 and 4 were fed on 0.05% choline, and by figures 5 and 6 on 0.10% choline. These pigs were in the ad libitum feeding test.
PLATE 2

EXPLANATION OF FIGURES

The figures on the left of the plate, viz., 7, 9, and 11, are photographs of pigs on the 0.05% choline level, whereas those on the right are their pair mates which were fed 0.10% choline. These pigs are typical of the pair-fed test.
Photomicrographs of liver sections of pigs at termination of experiment (8 weeks). Magnification × 225. All sections were made by paraffin method and stained with Harris's hematoxylin and Orange G.

13 Liver from pig on 0.0% level of choline (see fig. 2, plate 1).
14 Liver from pig on 0.05% level of choline (see figs. 3 and 4, plate 1). Note small amount of fatty infiltration.
15 Liver from pig on 0.10% level of choline (see figs. 5 and 6, plate 1). Note absence of abnormal fatty infiltration.
16 Liver from pig on 0.05% level of choline from paired feeding test (see figs. 7, 9, and 11, plate 2). Note small amount of fatty infiltration.
17 Liver from pig on 0.10% level of choline from paired feeding test (see figs. 8, 10 and 12, plate 2).
PLATE 4
EXPLANATION OF FIGURES

Photomicrographs of kidney sections of pigs at termination of experiment (8 weeks). Magnification × 75. All sections were made by paraffin method and stained with Harris's hematoxylin and Orange G.

18 Kidney from pig on 0.0% level of choline. Note extensive pathology of glomeruli and tubules.
19 Kidney from pig on 0.05% level of choline. Note some normal and some pathological glomeruli and tubules.
20 Kidney from pig on 0.10% level of choline. Note absence of pathological condition.
21 Kidney from pig of same age fed on an adequate farm ration. Note similarity to figure 20.
22 Kidney from pig on 0.05% level of choline from paired feeding test. Note same condition as found in figure 18.
23 Kidney from pig on 0.10% level of choline from paired feeding test. Note normal condition and similarity to figures 20 and 21.
CHOLINE REQUIREMENT OF PIG

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PLATE 4

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PLATE 5

EXPLANATION OF FIGURES

Photomicrographs of same kidney sections as shown in plate 4. Higher magnification ×225 has been used to show greater glomerular and tubular detail.