15481 P

Further Evidence for Methylatable Precursors of Choline in Natural Materials.*

H. L. Lucas,† L. C. Norris, and G. F. Heuser.

From the Agricultural Experiment Station and School of Nutrition, Cornell University, Ithaca, N.Y.

In the course of work on choline metabolism in chicks the choline content of several diets was determined by both a colorimetric method and a microbiological method. The colorimetric method was essentially that of Thornton and Browne[1] using reineckate precipitation. Ethanol was used to extract the choline-bearing substances from the original materials. Hydrolysis was carried out with 3% H_2SO_4 and the precipitation was done after neutralization to pH 6.0 with solid $Ba(OH)_2$ and then to pH 7.5 with 1 N NaOH. The microbiological method was that of Horowitz and Beadle[2] using the Neurospora "choline-less" mutant 34486. The standards as well as unknowns were passed through the adsorption and elution steps. Reproducibility was poor with the Neurospora method on some samples. The results are shown in Table 1.

On the purified and semipurified diets the 2 methods were in close agreement. On the practical diet with liver meal and the simplified diet, however, the Neurospora method gave substantially higher values. The simplified diet contained 15% peanut meal.

The value of >0.08% obtained for the simplified diet by the Neurospora method is in essential agreement with that published by McGinnis, Norris and Heuser.[3] The value of <0.04% obtained by the reineckate method is in agreement with a value calculated from the choline contents of the individual ingredients as published by Engel.[5]

The higher value obtained by Neurospora on the practical diet containing liver meal might be expected in view of the results of Jukes and Oleson.[6] These authors found that, if a crude aqueous extract of liver was hydrolized with $Ba(OH)_2$ and then precipitated with reineckate, the resulting filtrate was active for Neurospora.

Jukes and Dornbush[7] observed that dimethylaminoethanol stimulated the mutant Neurospora in a manner similar to choline. Apparently, however, dimethylaminoethanol was not precipitated by reineckate when pure and was only partially precipitated in the presence of choline. These results suggest that those diets on which the higher values were obtained by the Neurospora method in the present study contain dimethylaminoethanol or related compounds. Jukes and Oleson[8] have called attention to the fact that dimethylaminoethanol has been isolated from one member of the Leguminosae.

Numerous compounds were tested with 2 Neurospora "cholineless" mutants by Horowitz, Bonner and Houlahan.[9]. Their data

* This work was supported in part by grants from Commercial Solvents Corporation, Terre Haute, Ind., the Cooperative G.L.F. Exchange, Inc., Ithaca, N.Y., and Lederle Laboratories, Inc., Pearl River, N.Y. The experimental work was conducted in the laboratories of the Department of Poultry Husbandry. The assistance of Mrs. Blossom Branton in conducting the analyses is gratefully acknowledged.

† Now with the Institute of Statistics, University of North Carolina, Raleigh, N.C.

1 Thornton, M. H., and Browne, F. K., *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 39.

2 Horowitz, N. H., and Beadle, C. W., *J. Biol. Chem.*, 1943, **150**, 325.

3 McGinnis, J., Norris, L. C., and Heuser, G. F., Proc. Soc. Exp. Biol. and Med., 1944, **56**, 197.

4 Lucas, H. L., Norris, L. C., and Heuser, G. F., *Poultry Science*, 1946, **25**, 373.

5 Engel, R. W., *J. Nutrition*, 1943, **25**, 441.

6 Jukes, T. H., and Oleson, J. J., *J. Biol. Chem.*, 1945, **157**, 419.

7 Jukes, T. H., and Dornbush, A. C., Proc. Soc. Exp. Biol. and Med., 1945, **58**, 142.

8 Jukes, T. H., and Welch, A. D., *J. Biol. Chem.*, 1942, **146**, 19.

9 Horowitz, N. H., Bonner, D., and Houlahan, M. B., *J. Biol. Chem.*, 1945, **159**, 145.

TABLE I.
The Choline Content of Various Materials as Determined by the Reineckate and Neurospora Methods.

Material	Choline content, %	
	By reineckate	By Neurospora*
Purified diet†	<0.03	<0.04
Semipurified diet‡	<0.03	<0.04
Simplified diet§	0.04	>0.08
Peanut meal	0.21	>0.26
Range of 5 practical diets	0.10-0.13	0.11-0.15
Practical diet plus 5% liver meal	0.17	0.26

* The values preceded by the greater-than sign are lower limits of ranges having higher mean values.
† Diet 661 of McGinnis and co-workers.[3]
‡ Diet of Lucas and co-workers.[4]
§ Diet 543 of McGinnis and co-workers.[3]

indicate that, except for those compounds which are determined as choline in the reineckate method, the only compounds of significant effectiveness for Neurospora are mono- and dimethylaminoethanol and monoethylcholine.

On the simplified diet here studied, McGinnis and coworkers[3] observed that choline, methionine, and betaine prevent perosis, but on the purified diet choline only was found to be effective. They concluded that choline *per se* is required for the prevention of perosis. The results of Jukes and coworkers[6,9,10] indicate that mono- and dimethylaminoethanol and mono- and diethylcholine have choline activity for the chick. Mono- and dimethylaminoethanol may function in the chick as choline precursors since these workers found that methionine augmented materially the effects of these compounds.[10]

McGinnis and coworkers[3] have suggested that precursors of choline are present in the simplified diet on which methionine and betaine prevent perosis. Jukes and Oleson[6] have suggested that simple precursors of choline might exist in natural foods. The observations reported in the present paper support these views.

[10] Jukes, T. H., Oleson, J. J., and Dornbush, A. C., *J. Nutrition*, 1945, **30**, 219.

15482

Changes in Alkaline Phosphatase of Kidney Following Renal Damage with Alloxan.

MAUD L. MENTEN AND MILES JANOUCH.
From the Department of Pathology, University of Pittsburgh, and Children's Hospital, Pittsburgh, Pa.

The alkaline phosphatase which occurs in high concentration in the proximal convoluted tubules of the kidney rapidly disappears during degenerative changes in these structures. The enzyme has been shown to be reduced by nephrotoxic drugs. Alloxan in addition to its injurious action on the islets of Langerhans causes a varying degree of parenchymatous degeneration of the renal tubules and evidence is here reported of an associated depletion of renal alkaline phosphatase in the damaged kidney.

White rats are readily susceptible to alloxan. Bailey, Bailey and Leech[1] produced

[1] Bailey, C. C., Bailey, O. T., and Leech, R. S., *New Eng. J. Med.*, 1944, **230**, 534.