diets containing 8 B complex factors, including p-aminobenzoic acid and inositol, do not afford adequate nutrition if sulfonamides are included. This offers evidence for the contention[11] that another factor of the B complex is involved, particularly as liver extracts will correct this dietary inadequacy. The potentialities of toxicity of the sulfonamide in the diet led us to attempt to produce a similar condition using another intestinal antiseptic, aseptoform, the methyl ester of p-hydroxybenzoic acid. Stock diets containing 2% aseptoform were fed to rats and mice with no evidence of toxicity. Aseptoform added at 2% and at 0.6% to synthetic diets containing 8 B complex factors resulted in the development of the characteristic deficiency syndrome in rats on this diet. There being no interrelationship between p-aminobenzoic acid and aseptoform, this type of diet seemed superior to a sulfonamide and p-aminobenzoic acid-containing diet. The syndrome in rats produced by the inclusion of either aseptoform or sulfonamide in the diet is essentially the same. It seems, therefore, improbable that the factor of toxicity enters the picture. It is suggested that another factor exists which is synthesized by the bacteria of the gastro-intestinal tract when rats are placed on synthetic diets, or which is present in stock diets of natural foods, with the result that rats on these diets supplemented by sulfonamide or aseptoform do not develop a deficiency.

*Summary.* Sulfanilamide, replacing p-aminobenzoic acid in a synthetic diet containing 7 B complex factors including inositol, prevents the development of the syndrome heretofore associated with a deficiency of p-aminobenzoic acid, but it does not entirely replace p-aminobenzoic acid.

Intestinal antiseptics (sulfonamides and aseptoform) added to synthetic diets containing 8 B complex factors make possible the demonstration in rats of a deficiency syndrome which it is suggested may be due to a ninth B complex factor.*

* Preliminary results indicate biotin is not the factor.

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## Nicotinic Acid in Chick Nutrition.*

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The activity of nicotinic acid in the prevention and cure of canine blacktongue and human pellagra is well established. There has been much speculation, however, as to the importance of this vitamin in the diet of the chick and rat.

Mickelsen, Waisman, and Elvehjem[1] and Dann and Subbarow[2] have demonstrated that nicotinic acid is inactive in preventing or curing chick dermatitis, previously described as chick pellagra by Ringrose, Norris and Heuser.[3] This antidermatitis factor was later shown to be pantothenic acid by Wool-

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1 Mickelsen, O., Waisman, H. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1938, **124**, 313.

2 Dann, W. J., and Subbarow, Y., *J. Nutr.*, 1938, **16**, 183.

3 Ringrose, A. T., Norris, L. C., and Heuser, G. F., *Poul. Sci.*, 1931, **10**, 166.

4 Woolley, D. W., Waisman, H. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1939, **129**, 673.

ley, Waisman, and Elvehjem[4] and by Jukes.[5] In a more recent paper Waisman and Elvehjem[6] reported that variable results with chicks were obtained when nicotinic acid was added to a modified Goldberger ration, growth responses being noticed in several groups. The authors concluded, however, that the ration did not lend itself for the demonstration that the chick requires this vitamin.

In our work with the chick, using purified rations, we had occasionally noted small growth responses due to the addition of nicotinic acid. Upon further study we have been able to construct a ration fairly complete in all the necessary factors and yet sufficiently low in nicotinic acid to permit the study of the role of this vitamin in chick nutrition. We have observed in the absence of nicotinic acid a depression in the growth rate and the appearance of a deficiency condition similar to canine blacktongue.

The basal ration, 486N, had the following composition per 100 g: dextrin 61 g, alcohol extracted casein 18 g, gelatin 10 g, salts IV[7] 5 g, Ca $HPO_4$ 1 g, soy bean oil 5 g, eluate fraction $\cong$ 5 g of solubilized liver,[8] *l*-cystine 300 mg, thiamine HCl 300 $\gamma$, riboflavin 600 $\gamma$, biotin concentrate (S.M.A. No. 1000) 15 $\gamma$, pyridoxin HCl 400 $\gamma$, Ca pantothenate 1.5 mg, choline Cl 150 mg, *i*-inositol 100 mg, 2-methyl-1, 4-naphthoquinone 500 $\gamma$, and $\alpha$-tocopherol 300 $\gamma$. Each chick received 3 drops of a vitamin A and D concentrate weekly. A microbiological assay of this basal ration† showed a nicotinic acid content of 0.3 mg per 100 g of ration.

Day-old white Leghorn chicks were divided into groups of 6 and placed in electrically heated cages with raised screen bottoms. The experimental rations, prepared weekly, and water were supplied *ad libitum*. Crystalline nicotinic acid (Merck) was used throughout.

TABLE I.
Effect of Supplementary Nicotinic Acid on Growth of Chicks.

| Supplement to basal ration (486N) Mg nicotinic acid per 100 g | No. of chicks | No. dead | Wt at 3 wks g | Wt at 4 wks g |
|---|---|---|---|---|
| No supplement | 18 | 1 | 106 | 146 |
| 0.5 | 6 | 0 | 123 | |
| 1.0 | 6 | 0 | 153 | |
| 1.5 | 6 | 1 | 164 | |
| 2.0 | 6 | 0 | 163 | |
| 2.5 | 11 | 0 | 164 | |
| 5.0 | 6 | 0 | 173 | |
| 10.0 | 18 | 0 | 163 | 226 |

The results are summarized in Table I. On the basal ration the chicks grew at a relatively slow rate, weighing 106 g at 3 weeks of age. With chicks receiving higher levels of nicotinic acid the growth rate correspondingly increased until a level of 1.5 mg per 100 g of ration was reached. These chicks weighed 164 g at 3 weeks, 58 g better than the negative control groups. Higher levels did not appreciably increase the growth rate. Therefore, since the basal ration contained 0.3 mg of nicotinic acid per 100 g, the minimal level required was approximately 1.8 mg per 100 g of ration.

Beginning at about 2 weeks of age the entire mouth cavity of the deficient chicks, as well as the upper portion of the esophagus and the crop, became distinctly inflamed with a deep red color; however, the tip of the tongue appeared nearly white. The mouths of the control chicks, in contrast, remained a normal pink. A decrease in food consumption was also noticed. Upon the addition of nicotinic acid to the ration, food consumption and growth increased and the chick "blacktongue" symptoms disappeared within a few days. A lower supplementary level of nicotinic acid (0.5 mg per 100 g of ration) was required for the prevention of "blacktongue" symptoms than for maximal growth. Nicotinic acid amide had the same activity as nicotinic acid in curing deficiency symptoms.

5 Jukes, T. H., *J. Am. Chem. Soc.*, 1939, **61**, 975.

6 Waisman, H. A., and Elvehjem, C. A., *J. Nutr.*, 1940, **20**, 519.

7 Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **138**, 459.

8 Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, 1941, **141**, 521; Mills, R. C., Briggs, G. M., Jr., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 186.

† We wish to thank Mr. L. J. Teply for this assay.

Dann and Handler[9] and Snell and Quarles[10] have given evidence for the occurrence of a marked increase in the nicotinic acid content of the incubating egg and have suggested, therefore, that the hatched chick does not require a dietary source of this vitamin. From our experiments we may conclude that if such synthesis of nicotinic acid does exist in the growing chick there is not sufficient formation to supply enough of the vitamin for optimal growth and for the prevention of chick "blacktongue." The question of the synthesis of nicotinic acid by the chick can only be answered by further experimentation.

*Summary.* The growing chick requires a dietary source of nicotinic acid for optimal growth, and for the prevention of chick "blacktongue" as shown by the use of highly purified rations. The minimal level of this vitamin needed is approximately 1.8 mg per 100 g of ration.

9 Dann, W. J., and Handler, P., *J. Biol. Chem.*, 1941, **140**, 935.

10 Snell, E. E., and Quarles, E., *J. Nutr.*, 1941, **22**, 483.

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## Effect of Liver Damage on Urinary Morphine Excretion.

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Gross, Plant and Thompson[1] reported that liver damage by chloroform caused an increased urinary excretion of morphine in both tolerant and non-tolerant dogs. At the time of this report the presence of conjugated morphine was not recognized. Gross and Thompson[2] demonstrated that dogs excreted morphine in a combined form, and Oberst[3] in the same year showed that man also excreted a combined form of morphine. The excretion of combined morphine has shown that morphine undergoes far less destruction in the animal body than formerly supposed. While the nature of the combined morphine excretion product is still unknown, Oberst[4] believes that the morphine is conjugated with glucuronic acid. He has further demonstrated that the conjugation occurs with the hydroxyl groups and that both the phenolic and alcoholic hydroxyls may be conjugated.

Thompson and Gross[5] studying the combined morphine excretion compound in dog urine found that in this animal the combined morphine is excreted in two forms, namely a fraction which is easily hydrolyzed and the other which hydrolyzes only with more drastic procedure.

With the information that liver damage caused an increase in the excretion of free morphine in dogs, a study of the effect of liver damage on the combined morphine was undertaken in both tolerant and non-tolerant dogs.

Five non-tolerant and 3 tolerant animals were used in these experiments. The dogs were maintained on a constant diet and water intake. Morphine determinations were made before and after liver damage on a constant dose of morphine. As an indication of hepatic damage bromsulphalein dye determinations were also made before and after the liver damage. Five mg per kg of the dye were injected intravenously and the dye in 1 cc of clear serum determined at 3, 8 and 15 min after the injection. Carbon tetra-

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2 Gross, E. G., and Thompson, V., *J. Pharm. and Exp. Therap.*, 1940, **68**, 413.

3 Oberst, F. W., *J. Pharm. and Exp. Therap.*, 1940, **69**, 240.

4 Oberst, F. W., *J. Pharm. and Exp. Therap.*, 1941, **73**, 401.

5 Thompson, V., and Gross, E. G., *J. Pharm. and Exp. Therap.*, 1941, **72**, 138.