hour. Specific evidence of nicotinic acid deficiency was sought in 2 other patients (G.S. and T.D.) whose urines were tested for the fluorescent factor F_2 shown by Najjar *et al.*[12] to be absent in nicotinic acid deficiency. These tests, performed on specimens obtained just before the thiamin injection, showed F_2 values of 7 and 20 units per hour respectively, values which preclude the possibility of nicotinic acid deficiency.

Thus, it would seem that nicotinic acid deficiency has not masked any thiamin deficiency in these paralytic patients, nor is nicotinic acid deficiency itself a prerequisite for the development of paralytic poliomyelitis.

It is possible that deficiencies of other B factors may contribute to the development of paralysis in this disease. In this connection it is of interest to call attention to recent evidence suggesting that in the nervous system of the pig certain changes attributed in other hosts to thiamin deficiency may be due to lack of other vitamin B factors such as pyridoxine and pantothenic acid.[13,14] Although the components of the B complex are usually found together in foodstuffs and the occurrence of a deficiency related to only one member of the vitamin B group is very rare,[15] it will, nevertheless, be necessary to determine the presence or absence of possible deficiencies of one or more of the other vitamin B factors before the relationship of the status of vitamin B nutrition to paralytic poliomyelitis can be completely evaluated.[16,17,18]

Summary. The results of thiamin excretion tests in 7 children having paralytic poliomyelitis did not differ in the main from those found in normal children. This finding indicates that the status of thiamin nutrition need not be a factor in determining whether infection by poliomyelitis virus shall result in the paralytic, or in one of the nonparalytic forms of the disease. Some evidence has also been obtained that nicotinic acid deficiency need not be a prerequisite for the development of the paralytic form of the disease. The role of other members of the vitamin B complex in relation to paralytic poliomyelitis remains to be investigated.

12 Najjar, V. A., Stein, H. J., Holt, L. E., Jr., and Kabler, C. V., *J. Clin. Invest.*, 1942, **21**, 263.

13 Wintrobe, M. M., Stein, H. J., Miller, M. H., Follis, R. H., Jr., Najjar, V. A., and Humphreys, S., *Bull. J. H. Hosp.*, 1942, **71**, 141.

14 Wintrobe, M. M., Miller, M. H., Follis, R. H., Jr., Stein, H. J., Mushatt, C., and Humphreys, S., *J. Nutrit.*, 1942, **24**, 345.

15 György, P., *Ann. Rev. Biochem.* (Stanford Univ., Calif.), 1942, **11**, 309.

16 McCormick, W. J., *Canad. Med. Assn. J.*, 1938, **38**, 260.

17 McCormick, W. J., *Med. Rec.*, 1942, **155**, 89.

18 Helms, K., *Med. J. Austral.*, 1941, **1**, 717.

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Effect of p-Aminobenzoic Acid when Added to Purified Chick Diets Deficient in Unknown Vitamins.*

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Ansbacher[1] has reported that p-aminobenzoic acid is a growth-promoting factor for the chick. The basal diet which he used was a vitamin K deficient ration consisting largely of wheat middlings and yellow corn heated at 110° for about one week. Hammond, Miller and McClure[2] found that p-aminobenzoic acid is necessary for the

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by grants from the Wisconsin Alumni Research Foundation and Swift and Company.

1 Ansbacher, S., *Science*, 1941, **93**, 164.

2 Hammond, J. C., Miller, D., and McClure, H. E., *Poultry Sci.*, 1942, **21**, 185.

normal development of the keel bones of chicks receiving diets deficient in phosphorus.

We wish to report, in this paper, studies on the growth-promoting activity of p-aminobenzoic acid and a possible mechanism for this activity.

Day-old White Leghorn chicks were divided as evenly as possible into groups of 6 and placed in electrically heated cages with raised screen bottoms. The basal diets, prepared weekly and fed *ad libitum*, are given in Table I. The two diets differ only as to

TABLE I.
Basal Diets.

	484K per 100 g	486K per 100 g
Dextrin	59	61
Casein (alcohol extracted)	18	18
Gelatin	10	10
Salts IV‡	5	5
$CaHPO_4$	1	1
Soybean oil	5	5
Kidney residue	2	
Biotin concentrate		15 γ
Cystine	0.3 g	0.3 g
Inositol	0.1 "	0.1 "
Thiamin	0.3 mg	0.3 mg
Pyridoxin	0.4 "	0.4 "
Riboflavin	0.6 "	0.6 "
Pantothenic acid	1.5 "	1.5 "
Choline	150 "	150 "
Nicotinic acid	10 "	10 "
2-methyl-1,4-naphthoquinone	500 γ	500 γ
Alpha-tocopherol	300 γ	300 γ
Vitamin A and D concentrate	3 dps/wk	3 dps/wk

‡ *J. Biol. Chem.*, 1941, **138**, 459.

the source of biotin; in one (484K) it is supplied as kidney residue† and in the other (486K) as a biotin concentrate (SMA Conc. 1000). We have shown that chicks receiving these diets grow slowly, feather poorly, and become anemic.[3] The addition to these diets of a factor, or factors, found in solubilized liver and in the "eluate fraction" of solubilized liver, is necessary for normal growth.

The effects of various supplements added to the basal diets are summarized in Table II. The feeding of 5 mg of p-aminobenzoic acid per 100 g of diet 484K resulted in better growth and feathering than that obtained with the basal diet alone. A higher level of p-aminobenzoic acid, 10 mg per 100 g, produced a smaller growth response. The feeding of solubilized liver or the "eluate fraction" of solubilized liver gave maximum growth and normal feathers. When p-aminobenzoic acid was fed with the "eluate fraction," no further increase in growth occurred.

When the more purified diet, 486K, was used, levels of 5 mg of p-aminobenzoic acid per 100 g had no effect on growth or feathering whereas levels between 7.5 mg and 15 mg per 100 g gave noticeable responses in both growth and feather formation. Again, a higher level of p-aminobenzoic acid, 20 mg per 100 g, was not as effective as the lower level. The addition of solubilized liver or the "eluate fraction" again produced normal growth and feathering. p-Aminobenzoic acid likewise produced a growth response when 1% whole liver substance was added to the basal diet.

These results suggested that p-aminobenzoic acid was necessary for the chick and was responsible, in part, for the growth-promoting effects of solubilized liver and of the "eluate fraction." However, an assay of solubilized liver which contains the unknown factor(s) for the chick, showed that it supplies only 5.2γ of p-aminobenzoic acid per 100 g of diet when fed at 2%. When a level of p-aminobenzoic acid was fed (10γ per 100 g of diet) which approximated the level supplied by 2% solubilized liver no appreciable gain over the basal was noted.

The more purified diet (486K) was found to contain only 18γ of p-aminobenzoic acid per 100 g. This amount, although low, exceeds by 3 to 4 times the amount present in the solubilized liver, and thus it would seem that p-aminobenzoic acid is not a specific growth factor for the chick unless it is needed at very low levels.

Because of the relatively high level of p-aminobenzoic acid required to give a growth response, it was thought that this

† The residue after exhaustive extraction of defatted kidney with water, 50% ethanol, and 50% methanol.

3 Mills, R. C., Briggs, G. M., Jr., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 186.

TABLE II.
Results of Addition of Various Substances to the Basal Diets.

Diet	No. of chicks	No. dead	Avg wt at 4 weeks	Feathers 0 = very poor 100 = very good
484K only	48	8	157	34
,, + 5 mg p-aminobenzoic acid per 100 g	6	0	227	60
,, + 10 ,, ,, ,, ,, ,, ,,	11	0	175	50
,, + 2% solubilized liver	30	0	257	93
,, + "eluate fraction" ≅ 5% sol. liver	18	1	260	89
,, + ,, ,, ≅ ,, ,, ,, + 10 mg p-aminobenzoic acid per 100 g	6	0	261	90
486K only	36	1	158	32
,, + 5 mg p-aminobenzoic acid per 100 g	6	2	146	40
,, + 7.5 ,, ,, ,, ,, ,, ,,	6	0	208	60
,, + 10 ,, ,, ,, ,, ,, ,,	24	1	202	47
,, + 12.5 ,, ,, ,, ,, ,, ,,	6	0	194	50
,, + 15 ,, ,, ,, ,, ,, ,,	6	0	199	65
,, + 20 ,, ,, ,, ,, ,, ,,	6	0	167	30
,, + 2% sol. liver	24	0	238	89
,, + "eluate fraction" ≅ 5% sol. liver	18	0	231	87
,, + 1% whole liver substance	6	0	215	70
,, + ,, ,, ,, ,, + 10 mg p-aminobenzoic acid per 100 g	6	0	246	85

compound causes the synthesis of the necessary unknown factor(s). The most logical manner of such production is through intestinal synthesis. To demonstrate that intestinal synthesis of the unknown factor(s) does occur in chicks receiving the basal diet (484K), an experiment was set up feeding sulfasuxidine (succinyl sulfathiazole) which diminishes the growth of certain intestinal organisms.[4] The results show (Table III) that growth and feathering on our basal diet may be retarded by at least 50% by the feeding of sulfasuxidine, indicating that intestinal synthesis of the unknown factor(s) is slowed down. That the drop in growth was not due to toxicity of the sulfasuxidine was shown by feeding it with the "eluate fraction" whereupon maximum growth was obtained.

In order to determine whether or not p-aminobenzoic acid can stimulate the growth of intestinal organisms and thereby, possibly, the synthesis of the unknown factor(s), the following *in vitro* experiment was set up: Tubes of sterile media (10 cc) containing various levels of p-aminobenzoic acid were inoculated with a mixed culture of organisms (obtained from the duodenum of chicks on the basal diet 1-4 weeks), incubated at 37° for 24-48 hr, and the amount of growth observed. The media, low in the unknown vitamin(s), has been described by Mitchell and Snell[5] for the determination of "folic acid" and was chosen for this purpose because the properties and occurrence of "folic acid"[6] are quite similar to the properties of

[4] Poth, E. J., and Knotts, F. L., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 129.

[5] Mitchell, H. K., and Snell, E. E., *Univ. of Texas Publication* No. 4137, 1941, 36 and 87.

TABLE III.
Effect of Sulfasuxidine on Chick Growth.

Supplement to basal diet 484 K	No. of chicks	No. dead	Avg wt at 4 weeks	Feathers 0 = very poor 100 = very good
No supplement	6	0	168	30
"Eluate fraction" ≅ 5% sol. liver	6	0	243	100
½% sulfasuxidine	5	3	73	15
"Eluate fraction" ≅ 5 sol. liver + ½% sulfasuxidine	6	0	252	100

TABLE IV.
In vitro Effect of Various Levels of p-Aminobenzoic Acid on Growth and "Folic Acid" Production of Intestinal Microörganisms from Chicks.

Gamma of p-aminobenzoic acid per tube	Turbidity reading	Folic acid content§ %
0	100	.030 (.018-.048)
10	98[3]	.031 (.023-.040)
30	98	.036 (.025-.048)
40	94[2]	.076 (.048-.104)
80	93	.101 (.092-.118)
100	94	.092 (.068-.124)

§ The figures of folic acid content represent the percentage activity as compared to a standard of solubilized liver.

the unknown factor(s).[3,7,8] No attempt was made to determine the various kinds of organisms contained in the mixed cultures. Growth was measured by turbidity readings in the Evelyn Photoelectric Colorimeter.

Results of a typical series are shown in Table IV. It may be seen that as the amount of p-aminobenzoic acid was raised, bacterial growth increased. This is not surprising since it is known that p-aminobenzoic acid is a growth factor for various microorganisms. In this series measurements of each tube for "folic acid," according to the Mitchell and Snell method[6] showed that as growth increased the synthesis of "folic acid" increased approximately 3-fold.

The significance of these *in vitro* studies is that p-aminobenzoic acid can stimulate "folic acid" production and growth of microorganisms found in the intestine of chicks. This would indicate that other unknown factor(s) may be produced and then when p-aminobenzoic acid is *fed* to chicks on diets low in such factor(s) it may also cause the production by intestinal synthesis of the unknown factor(s). Further work must be done before definite conclusions can be drawn.

In Table II it can be seen that although the two basal diets were similar the more purified diet (486K) required a greater amount of p-aminobenzoic acid to give a growth response than did diet 484K. Assays of both kidney residue and the biotin concentrate show that neither of them contain enough p-aminobenzoic acid (supplying 0.4γ and 2.4γ per 100 g respectively) to account for this difference. However, we can explain this difference on the assumption that the kidney residue affects the intestinal flora in a different manner than the biotin concentrate, and thus changes the concentration of p-aminobenzoic acid necessary in the intestine for maximum bacterial stimulation. It is also possible that the kidney residue contains some other factor which makes the p-aminobenzoic acid more effective.

Likewise, we can now explain the report of Ansbacher[1] in which he concluded that p-aminobenzoic acid was a factor of the vitamin B complex and that it was growth-promoting for the chick. The ration which he used was no doubt low in the unknown factor(s) since Waisman *et al.*[9] have shown that heated grain rations of this type are low in these factor(s). When the high level of p-aminobenzoic acid (30 mg per 100 g) was added to Ansbacher's ration, stimulation of bacterial growth in the intestine may have occurred as it did in our experiments.

Summary. p-Aminobenzoic acid at levels of 5 to 15 mg per 100 g of diet produces growth responses in chicks receiving purified rations low in the unknown vitamins but complete in all other respects. Evidence is given however to suggest that p-aminobenzoic acid is not a specific growth factor for the chick but may act indirectly by stimulating intestinal microörganisms to produce unknown factor(s) essential for the chick.

We are indebted to Merck and Company, Rahway, N.J., for the crystalline vitamins; to Wilson Laboratories, Chicago, Ill., for solubilized liver (liver fraction L), and whole liver substance; to Wilson and Co., Inc., Chicago, Ill., for gelatin; and to Allied Mills, Inc., Peoria, Ill., for soy bean oil.

We are grateful to Mr. L. J. Teply and Mr. J. O. Lampen for the p-aminobenzoic acid assays.

6 Mitchell, H. K., Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1941, **63**, 2284.

7 Hutchings, B. L., Bohonos, N., Hegsted, D. M., Elvehjem, C. A., and Peterson, W. H., *J. Biol. Chem.*, 1941, **140**, 681.

8 Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, 1941, **141**, 521.

9 Waisman, H. A., Mills, R. C., and Elvehjem, C. A., *J. Nutrition*, 1942, **24**, 187.