

the sixth treatment, the membrane was stroked with a moist cotton swab and with it half of a nutrient agar plate was streaked. The number of bacteria recovered on the petri plate was considered to be an indication of the degree of infection and was recorded as follows: 0 = no colonies; + = 1-10 colonies; ++ = more than 10, but plate not covered; +++ = plate covered with growth.

The results shown in Table I indicate that the infected chorio-allantoic membrane of the developing chick embryo may be used to demonstrate definite and decisive differences in the therapeutic value of disinfectants. These data represent the results of several experiments and since the results of

such tests were similar, one may conclude that the findings are readily reproducible. The method as described makes it possible to determine with accuracy the comparative value of disinfectants under conditions more comparable to infected wounds than can be obtained by any *in vitro* method now available.

Insofar as the disinfectants themselves are concerned, it is evident from the data reported that (1) phenol did not show any demonstrable beneficial action, (2) with the exception of one embryo, iodine had no therapeutic effect, and (3) cetyl pyridinium chloride had a definite curative effect on the experimental infection.

13825

p-Aminobenzoic Acid and Sulfonamides in Rat Nutrition.

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Nutritional experimentation involving p-aminobenzoic acid¹⁻⁵ and the sulfonamides^{6,7,11,12,13} has disclosed a similarity of action of the two agents. The role of both sulfonamides¹⁰ and p-aminobenzoic acid⁵ in altering the microflora of the intestine suggested the investigation under consideration in this report.

Three series of black rats were placed on diets with and without p-aminobenzoic acid and sulfanilamide.

Basal Diet.	
Vitamin free casein	18
Sucrose	67
Salts	4
Butter fat	9
Cod liver oil	2

Vitamin Supplements per kg of Diet.

	mg
Thiamine hydrochloride	5
Riboflavin	10
Pyridoxine	5
Nicotinic acid	100
Calcium pantothenate	100
Choline chloride	200
Inositol	200

Series 1: 20 black rats, weighing 40 to 50 g, were placed on the above diet containing the 7 B complex vitamins without p-aminobenzoic acid or sulfanilamide. On this diet, as previously reported,⁵ rats develop symptoms which terminate in death in 6 or 7

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³ Unna, K., Richards, G. V., and Sampson, W. L., *J. Nutr.*, 1941, **22**, 553.

⁴ Henderson, L. M., McIntire, J. M., Waisman, H. A., and Elvehjem, C. A., *J. Nutr.*, 1942, **23**, 47.

⁵ Martin, G. J., *Am. J. Physiol.*, 1942, **136**, 124.

⁶ Woolley, D. W., personal communication.

⁷ Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., *Science*, 1942, **94**, 518.

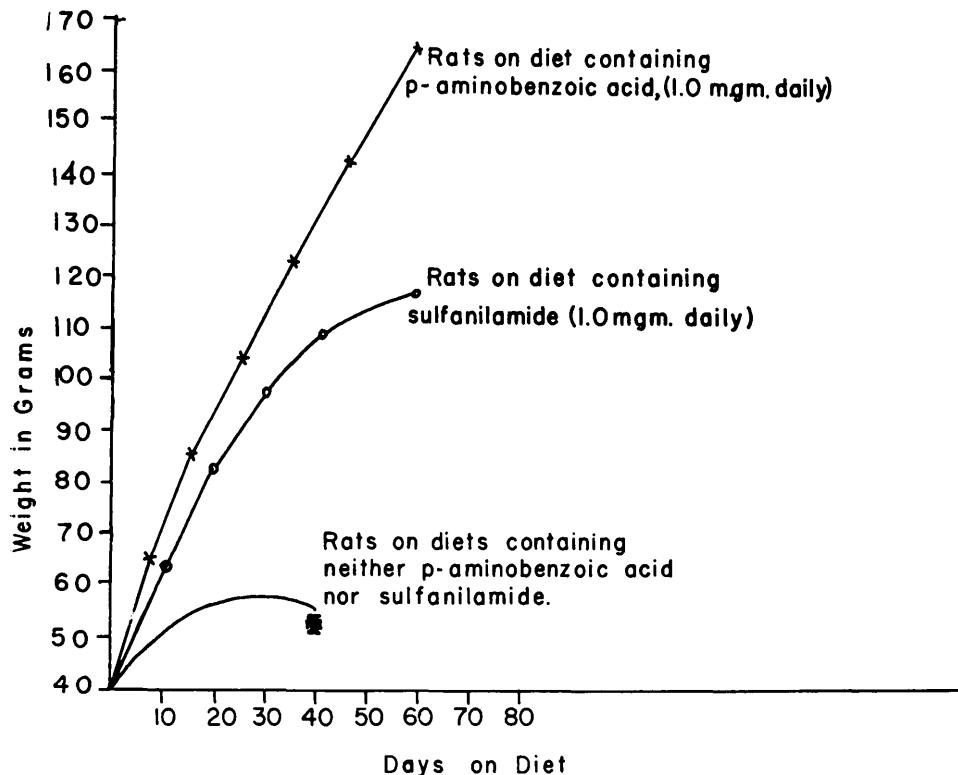
¹⁰ Marshall, E. K., Jr., Bratton, A. C., White, H. J., and Litchfield, S. T., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.

¹¹ Black, S., McKibbin, J. M., and Elvehjem, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1941, **47**, 308.

¹² Daft, F. S., Ashburn, L. L., Spicer, S. S., and

Sebrell, W. H., *U. S. Pub. Health Reports*, 1942, **57**, 217.

¹³ Welch, A. D., *Fed. Proc.*, 1942, **1**, 171.



THE EFFECT ON GROWTH OF RATS OF p-AMINO BENZOIC ACID AND SULFANILAMIDE IN SYNTHETIC DIETS.

weeks. The weights attained vary from 50 to 70 g. (Fig. 1).

Series 2: 20 black rats, weighing 40 to 50 g, were placed on a diet containing 7 of the vitamins listed, plus p-aminobenzoic acid (1 mg daily). These rats, as previously reported,⁵ seem normal in every respect except that the coats are brownish with a slight residual greying. The weights at 6 weeks range from 110 to 180 g. (Fig. 1). These animals, after prolonged periods on the diet (6 months to a year) develop an appearance suggesting hyperthyroidism. The eyes seem to protrude, the white of the eye being more than usually visible. It might be that this effect is produced by abnormal withdrawal of the eyelid, as it was found that these animals⁸ actually possess an atrophied thyroid and not a hypertrophied thyroid.

Series 3: 20 black rats, weighing 40 to

50 g, were placed on the synthetic diet containing 7 of the B complex factors and sulfanilamide (1 mg daily). At 6 weeks, these animals vary in weight from 90 to 160 g. They show coal black coats, and none of the brownishness or the slight residual greying seen in series No. 2 is evident in this group. At 10 weeks, the growth curves (Fig. 1) plateau, and some of the rats begin to lose weight gradually.

The experiments⁵ demonstrating the action of p-aminobenzoic acid in precipitating a syndrome prevented by inositol suggested the possibility that the syndrome associated with or produced by the inclusion of sulfonamides^{7,11,12,13} in synthetic diets was due to a similar action. Examination of the diets used by these investigators^{7,11,12,13} revealed the absence of inositol from any of the synthetic diets fed, and it seemed logical, therefore, to repeat these experiments and to extend them by adding inositol to the diet of one series.

⁸ Hueper, W. C., and Martin, G. J., unpublished data.

Series 4: 10 black rats (40 to 50 g in weight) were placed on the basal diet as described but receiving supplements of thiamine, riboflavin, pyridoxine, nicotinic acid, calcium pantothenate and choline chloride. If sulfanilamide or sulfaguanidine was added to this diet at 0.5%, the rats failed to grow and developed the characteristic⁵ syndrome of an inositol deficiency.

Series 5: 10 black rats (40 to 50 g in weight) were placed on the basal diet containing 8 B complex factors (the six listed above plus p-aminobenzoic acid and inositol), with sulfanilamide or sulfaguanidine present at 0.5%. Rats on this diet did not develop the syndrome associated with an inositol deficiency,⁵ but they did plateau in 60 days at weights markedly lower than those of Series 6. They develop a syndrome in which bleeding of the eyes, progressing to a severe panophthalmitis, is the most prominent symptom.

Series 6: 10 black rats (40 to 50 g in weight) were placed on our stock ration to which sulfaguanidine (2%) or sulfanilamide (1%) had been added. These animals grew normally and were normal in all macroscopic aspects.

In order to circumvent the complications inherent in the use of both sulfonamides and p-aminobenzoic acid in the same diet, the use of a different type of intestinal antiseptic seemed indicated. Aseptoform, the methyl ester of p-hydroxybenzoic acid, was selected because of its low toxicity.

Series 7: 10 black rats (40 to 50 g in weight) were placed on our stock diet supplemented by 2% aseptoform. Here again, as in the case of the sulfonamide stock diet, the animals grew normally and seemed normal in every respect.

Series 8: 10 black rats (40 to 50 g in weight) were placed on the synthetic diet previously described containing 8 B complex factors but with 2% aseptoform added. Animals on this diet die in 4 to 6 weeks, having attained weights of from 40 to 70 g. The most prominent symptoms are those of bleeding from the eyes, which progresses to a severe pan-ophthalmitis and a swollen edematous head.

Series 9: 10 black rats (40 to 50 g in weight) were placed on the same synthetic ration as series 8, but the aseptoform comprised only 0.6% of the ration. This group did not differ significantly from group 8 in growth performance or in symptomatology.

Discussion. Sulfanilamide replaces p-aminobenzoic acid to a considerable extent in the diet of the rat. The growth curve of rats on the diet containing sulfanilamide but no p-aminobenzoic acid plateaued at 10 weeks, while that of the p-aminobenzoic acid fed rats did not. When the sulfanilamide dosage was increased 1000%, the same growth curve was observed. The two agents, sulfonamide¹⁰ and p-aminobenzoic acid⁵ produce a similar effect: namely, the inhibition of the growth of certain microorganisms. Using either compound as a dietary component, the syndrome of an inositol deficiency can be produced, and the syndrome associated with or resulting from the inclusion of inositol in the diet can be prevented by p-aminobenzoic acid or partially prevented by sulfanilamide.

Sulfonamides added to stock diets in percentages as high as 5% do not significantly alter the nutritional behavior of the animal. Sulfonamides added to synthetic diets precipitate a syndrome of severe deficiency. Black *et al.*¹¹ found that liver extract, and to a lesser degree p-aminobenzoic acid, prevented the growth inhibiting effect of sulfaguanidine in rats fed a purified ration. These results were independently confirmed by Mackenzie *et al.*⁷ The investigators^{7,11} did not eliminate inositol as a factor in their diets. Sulfaguanidine at 1% added to a synthetic diet was found by Daft *et al.*¹² to result in the development of hyaline sclerosis and calcification of blood vessels in rats on this diet. Both inositol and p-aminobenzoic acid were absent from the diet of these investigators. Welch¹³ found no toxic manifestations and no depression of growth of rats when he added succinyl sulfathiazole at 5% to stock diets on which the rats were kept. Although p-aminobenzoic acid did not correct the syndrome developed when this sulfonamide was added to synthetic diets, liver did.

Our observations have demonstrated that

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 contention¹¹ 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.

13826

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.

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Mickelsen, Waisman, and Elvehjem¹ and Dann and Subbarow² 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-

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.