13507

Toxicity of *p*-Aminobenzoic Acid.

C. C. Scott and E. B. Robbins. (Introduced by K. K. Chen.)
From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis.

Recent investigations have shown that *p*-aminobenzoic acid is a substance of biological interest. Since this compound possesses a curative action for achromotrichia of rats and mice and is necessary for proper reproduction and lactation in rats, it appears to be a vitamin of the B complex.[1, 2, 3] In addition, the bacteriostatic action of the sulfonamides is inhibited by *p*-aminobenzoic acid.[4] Further, it probably is important for metabolism of plants in general.[5] In human subjects, darkening of gray hair has resulted from ingestion of this substance.[6] Because of these studies, the importance of toxicity determinations of *p*-aminobenzoic acid becomes evident.

Acute Toxicity. Mice, rats, and dogs were employed. For oral studies, the free acid was administered by stomach tube. The sodium salt was prepared for the intravenous toxicities since the free acid is too insoluble. *p*-Aminobenzoic acid is more toxic to dogs and mice than to rats. The median lethal dose ($LD_{50} \pm$ standard error) in mice was observed to be 2.85 ± 0.4, that in rats greater than 6, and that in dogs between 1 and 3 g per kg. It is interesting that the sodium salt of *p*-aminobenzoic acid, in contrast with the free acid, is more toxic to rats than mice by intravenous injection. No explanation is as yet available for this reversal of toxicity. The results are shown in Table I.

Orally, toxic signs in mice consisted only of weakness and loss of normal posture, death occurring in several hours. Mild clonic convulsions with death in 5-10 minutes resulted from intravenous lethal doses in mice and rats.

In dogs, 1-2 days passed before death occurred. Toxic signs were tremors, listlessness, and weakness; 2 animals vomited; and tonic and clonic convulsions were observed in the dog which received the largest dose. Autopsies on the animals which received lethal doses showed acute gastro-enteritis with hemorrhages apparently of cap-

1 Ansbacher, S., *Science*, 1941, **93**, 64.
2 Martin, G. J., and Ansbacher, S., *J. Biol. Chem.*, 1941, **138**, 441.
3 Sure, B., *Science*, 1941, **94**, 167.
4 Woods, D. D., and Fildes, P., *J. Soc. Chem. Ind.*, 1940, **59**, 133.
5 Wiedling, S., Science, 1941, **94**, 389.
6 Sieve, B. F., *Science*, 1941, **94**, 257.

TABLE I.
Acute Toxicity of *p*-Aminobenzoic Acid.

Substance	Animal	Administration	Dose, g per kg	No. died / No. used	$LD_{50} \pm$ standard error g per kg
Free Acid	Mouse	Oral	2.5	1/5	2.85 ± 0.4
			3.0	5/8	
			3.5	9/10	
			4.0	8/8	
	Rat		2.0	0/8	>6.0
			2.5	0/7	
			3.0	0/7	
			3.6	0/8	
			4.2	0/8	
			5.0	0/8	
			6.0	0/8	
	Dog		0.5	0/3	between 1.0
			1.0	1/3	and 3.0
			1.5	1/1	
			2.0	1/1	
			3.0	1/1	
Sodium Salt	Mouse	Intravenous	3.0	1/13	4.60 ± 0.21
			4.0	1/13	
			5.0	11/18	
			6.2	8/8	
	Rat		1.6	0/5	2.76 ± 0.24
			2.0	0/5	
			2.4	2/5	
			3.2	3/3	
			4.0	4/5	

illary origin in the small intestine. Acute necrosis of the liver occurred in the dogs given the two highest dosages.

Feeding Experiments. By stomach tube, 6 rats received 0.6, and 7 rats 1.4 g per kg of *p*-aminobenzoic acid daily for 28 days. As controls, 7 rats were given 1.5 cc of water by stomach tube for the same period. All the animals were fed a standard stock diet. The rats were 6 weeks old when medication was started. Throughout the experimental period, the gain in weight for all 3 groups was the same. Autopsies on all of these animals were entirely negative. It is evident from these chronic studies, as well as the acute toxicities, that the rat is very resistant to *p*-aminobenzoic acid.

Summary. 1. In mice, rats, and dogs, the acute toxicity of *p*-aminobenzoic acid by mouth has been determined. The drug is more toxic to mice and dogs than to rats. By intravenous injection the sodium salt of *p*-aminobenzoic acid is more toxic to rats than to mice. 2. Oral doses of *p*-aminobenzoic acid greater than 1.0 g per kg to dogs may cause death accompanied by acute gastro-enteritis

and hemorrhages into the small intestine. Acute necrosis of the liver may result from doses of 2.0 g per kg or larger. 3. Rats tolerate daily doses of 1.4 g per kg by mouth for about a month without inhibition of growth or pathological changes.

13508

Lactobacillus casei ε Factor in the Nutrition of the Chick.*

R. C. MILLS, G. M. BRIGGS, JR., C. A. ELVEHJEM AND E. B. HART.

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wisconsin.

The activity of a norit eluate fraction required by *Lactobacillus casei* ε, in promoting growth in chicks receiving purified rations, was recently reported from this laboratory.[1] Further studies have been made on the rôle of this fraction in chick nutrition and the results are presented here. It has been found that the *Lactobacillus casei* ε eluate fraction contains a factor or factors essential for growth, hemoglobin formation, and normal feathering in growing chicks.

The basal ration used had the following percentage composition: dextrin 59, purified casein 18, cartilage 10, kidney residue 2, salts IV[2] 5, $CaHPO_4$ 1, and soybean oil 5. Each kg of ration also contained thiamin 3 mg, riboflavin 4 mg, pyridoxine 4 mg, Ca pantothenate 15 mg, nicotinic acid 100 mg, choline 1.5 g, and inositol 1 g. Each chick received 2 drops of haliver oil per week. In more recent work 10% gelatin and 0.2% cystine were substituted for cartilage in order to produce a more deficient ration. It has been found that gelatin and cystine can replace cartilage in promoting growth.[3]

Day-old White Leghorn chicks were obtained either from a commercial hatchery or from the University poultry department. They

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. We are indebted to Merck and Company, Rahway, N.J., for the synthetic vitamins; to Abbott Laboratories, North Chicago, Ill., for haliver oil; to Wilson Laboratories, Chicago, Ill., for cartilage and solubilized liver extract; and to Allied Mills, Peoria, Ill., for soybean oil.

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

2 Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **138**, 458.

3 Unpublished data.