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Toxicity of Vitamin B_6.

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The existence of vitamin B_6, the rat acrodynia factor was first established by György.[1] The compound has since been isolated[2-5] and synthesized.[6, 7] Sufficient amounts of the synthetic vitamin, 2-methyl-3-hydroxy-4,5-dihydroxymethylpyridine, are now available for the study of the effect of large doses in animals.

The following investigation of the toxicity of the synthetic vitamin B_6 has been carried out on rats, dogs, and monkeys. All animals were maintained on completely adequate diets with the exception of one series of rats which was kept on a modified Sherman-Spohn diet supplemented with 40 micrograms of thiamin and of riboflavin per rat per day. This group developed typical symptoms of rat acrodynia.

The vitamin was administered both as the base and as the hydrochloride. The solutions of the base are neutral in reaction; those of the hydrochloride react acid, the pH of a 20% solution being 2.3.

Acute toxicity following oral and subcutaneous administration was studied in rats, 10 animals being used for each dose level. Doses up to 1 g per kg were tolerated without untoward effects. Higher doses produced rather peculiar toxic symptoms. Twenty-four hours after dosing, the rats showed tonic convulsions, the hind limbs stretched away from the body, the fore limb bent under the body and the paws closed. Between convulsive attacks the animals were able to move slowly and awkwardly and showed marked impairment of the righting reflexes. Animals receiving sublethal doses exhibited these convulsive reactions over a period extending from several days to as much as 3 weeks. With lethal doses the animals died in tonic convulsions within 36 to 72 hours. The L.D. 50* following subcutaneous injection was 3.1 g per kg for the free base and 3.7 g

1 György, P., *Biochem. J.*, 1935, **29**, 760.

2 György, P., *J. Am. Chem. Soc.*, 1938, **60**, 983.

3 Keresztesy, J. C., and Stevens, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 64.

4 Kuhn, R., and Wendt, G., *Ber.*, 1938, **71**, 780.

5 Lepkovsky, S., *Science*, 1938, **87**, 168.

6 Harris, S. A., and Folkers, K., *Science*, 1939, **89**, 347.

7 Kuhn, R., Wendt, G., Westphahl and Westphahl, K., *Naturwissenschaften*, 1939, **27**, 469.

* L.D. (lethal dose) 50 is the dose which is fatal to 50% of the animals.

per kg for the hydrochloride. This difference of 16% in toxicity is in good agreement with the difference of 18% in the molecular weights of the respective compounds. The L.D. 50 following oral administration was approximately 4 g per kg for the base and 6 g per kg for the hydrochloride. Autopsies of animals exhibiting various degrees of toxic manifestations failed to show pathologic changes on gross examination except for enlargement of the adrenals with occasional massive hemorrhages particularly in the cortex. Histological studies are in progress.

Three-week-old rats in groups of 15 were fed orally 0.25, 1.0, and 2.5 mg of B_6 hydrochloride daily over a period of 87 days. The animals developed normally and their weights increased at the same rate as that of the normal control group. Autopsies at the end of the 87-day feeding period showed no gross or microscopic changes in the organs. A group of 6 animals, 3 males and 3 females, were kept to maturity on a daily dose of 2.5 mg of the hydrochloride. Two litters, one of 5 and the other of 8, averaging 5 g birth weight, were obtained.

In dog studies 20 mg of B_6 per kg body weight was given orally to 3 litter mate puppies over a period of 75 days. Their weight increase was normal and did not differ from that of a control dog from the same litter. Hemoglobin, erythrocyte, leukocyte, and differential blood counts were taken at regular intervals and did not show any significant changes. Histological examination after 75 days revealed no pathological changes.

Monkeys of 2.5 and 4 kg body weight were fed or injected daily with 10 mg per kg B_6 hydrochloride. Unfortunately, most of the animals succumbed to tuberculosis during the test period. One monkey was dosed orally for 39 days, another subcutaneously for 106 days without showing any toxic symptoms. Hemoglobin and blood cell counts as well as the differential picture of the sternal bone marrow obtained by puncture did not fluctuate more than in a control monkey during the same period.†

Eight rats in severe stages of vitamin B_6 deficiency, weighing between 40 and 50 g, were given 9 mg of B_6 hydrochloride orally on two consecutive days. This dose is far in excess of the minimum curative dose of 50 micrograms.[8] Prompt cures were effected, and no toxic symptoms were observed.

† We are grateful to Dr. Lester Goldman for these data which will be presented in full in another communication.

[8] Reedman, E. J., Sampson, W. L., and Unna, K., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 112.

The results of these studies characterize vitamin B_6 as a substance of very low toxicity corresponding with the low toxicity found for other members of the vitamin B complex, thiamin,[9] riboflavin,[10] and nicotinic acid.[11] Excessively large doses (3.0 g per kg) of vitamin B_6 produced convulsions and death.

The difference in subcutaneous and oral toxicity, as shown by the L.D. 50, is small and suggests a rapid and complete absorption from the intestinal tract.

Prolonged feeding of sublethal doses failed to produce toxic symptoms, thus indicating that excessive doses of vitamin B_6 are either rapidly excreted or destroyed. Studies on this problem are now in progress.

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Urinary Excretion of Vitamin B_6 in the Rat.

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(Introduced by H. Molitor.)

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Following the isolation,[1] identification,[2] and synthesis[3] of vitamin B_6, as reported from these laboratories, the urinary excretion of the vitamin has been studied in the rat.

Recently, Kuhn and Low[4] reported on the use of the Folin-Denis reagent for the colorimetric determination of the vitamin in aqueous solutions. Stiller, Keresztesy and Stevens[2] observed that the vitamin gave a positive Gibbs reaction.[5] This reaction has been modified and

9 Molitor, H., and Sampson, W. L., *E. Merck's Jahresber.*, 1936, **50**, 51.

10 Kuhn, R., *Klin. Wchnschr.*, 1938, **17**, 222.

11 Unna, K., *J. Pharm. and Exp. Therap.*, 1939, **65**, 95.

1 Keresztesy, J. C., and Stevens, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 64.

2 Stiller, E. T., Keresztesy, J. C., and Stevens, J. R., *J. Am. Chem. Soc.*, 1939, **61**, 1237; Harris, S. A., and Folkers, K., *J. Am. Chem. Soc.*, 1939, **61**, 1242.

3 Harris, S. A., and Folkers, K., *J. Am. Chem. Soc.*, 1939, **61**, 1245.

4 Kuhn, R., and Low, I., *Ber.*, 1939, **72**, 1453.

5 Gibbs, H. D., *J. Biol. Chem.*, 1927, **72**, 649.