Toxicity of Pantetheine.* (23212)

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Pantothenic acid has been shown to prevent achromotrichia in animals. The results, however, are not consistent and very high levels of the vitamin are sometimes required. The metabolically active forms of pantothenic acid are coenzyme A and pantetheine. It is probable that the inconsistency among various experiments may be due to certain uncontrolled factors. For example, rate of formation of pantetheine from pantothenic acid may be limiting in some species.

To study antigray-hair activity of pantetheine, a systematic study of its toxicity is required. As described below, pantetheine is relatively innocuous, with LD_{50} of 5 to 7.5 g/kg body weight of mice.

Methods. The pantetheine was either synthesized in this laboratory(1) or purchased from Krishell Laboratories. Solutions containing 100 to 150 mg of pantetheine and 30 mg glucose ml in 0.02 M phosphate buffer, pH 6.2, were distributed into 10 ml ampules and sterilized for 30 minutes at 100°C, then stored in a refrigerator. Solutions for control experiments contained all ingredients except pantetheine. In experiments for chronic toxicity, the solutions were diluted 10 fold with sterilized saline solution to obtain a desirable injection volume, about 0.3 ml/animal. Rabbits were kept in individual cages. Mice of both sexes were caged in 2 groups, *i.e.*, control and experimental. Stock diets were used throughout. All animals and diets were kindly supplied from the Animal Husbandry Department of this college.

Results. Two rabbits, weighing 9 and 10 lb, received intravenous daily doses of 0.5 g pantetheine/animal for 4 days. No ill effect was observed.

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Acute toxicity and LD_{50} in mice. The LD_{50} test for pantetheine in mice by intraperitoneal injection was conducted as shown in Table I. It is evident that the LD_{50} lies between 5 and 7.5 g pantetheine/kg body weight of mice. At 5 g/kg the mice showed some immediate signs of mild depression but only one animal died. When a 7.5 g/kg dose was administered, 12 animals out of 15 died within 4 hours and another within 20 hours. Two animals survived. General depression and apparent muscular incoordination was observed among most animals. At the 10 g/kg level most animals died within an hour. Within 20 minutes after administration of pantetheine, signs of depression were observed as expected. No such abnormalities were shown among the control animals receiving the same volumes of the solution without pantetheine.

Chronic toxicity. The mice were divided into 2 groups of 51 animals each. Cages were changed 5 times a week after animals were injected and fed. Weighings were made before injection. Two-tenths g of pantetheine/kg body weight was administered during these experiments. Both control and experimental animals received 5 injections each week, Monday through Friday, for 10 weeks. During the first 3 weeks, 5 animals in the control group and 4 in the experimental group died.

TABLE I. Acute Toxicity of Pantetheine in Mice.

Dose, g/kg*	Avg vol of inj., ml	Avg individual wt of mice, g	No. of animals tested	No. of animals died (24 hr)
2	.32	16	13	0†
5	.97	19.5	13	1
7.5	.94	18	15	13
10	1.18	17.2	12	12
0	.35	17.9	11	0†
0	1.39	18.4	11	0†

* In g of pantetheine/kg body wt.
† No death after 1 wk.

After that time, no deaths were observed. The controls appeared to have a higher average mean weight, namely about 2%, than did the experimental animals based on the first weighing.

However, the experimental group contained 29 females and 22 males whereas the control group contained 23 females and 28 males. The apparent higher gain in body weight in the control group could be attributed to unequal sex distribution in these 2 groups. Thus the *ratio* of mean weight of the experimental animals to that of the controls was calculated and was practically constant at about 0.96. The increase in ratio from the 44th injection onward was due to pregnancy of the animals since more females were in the experimental group. It is concluded that administration of pantetheine at 0.2 g/kg did not affect growth of mice under the conditions tested.

Summary. The LD_{50} of pantetheine in mice was found to lie between 5 and 7.5 g/kg body weight. General depression, and eventual death were observed in animals after administration of high dosages of the compound. Intraperitoneal injections of pantetheine at 0.2 g/kg body weight continuously for 10 weeks did not affect growth of mice.

1. King, T. E., Stewart, C. J., and Cheldelin, V. H., *J. Am. Chem. Soc.*, 1953, v75, 1290.

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Amylase Clearance and Excretion During Water Diuresis.* (23213)

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The effect of increasing urinary flow on urea clearance values(1,2) has been well established. Urea clearances rise rapidly at low urine flows up to 0.35 ml/min. but above this rate of urine flow the increase in clearance is slight. Inulin and PAH clearances in man(1,3) do not seem to be influenced by diuresis. However, data on effect of diuresis on protein excretion and clearance are lacking. In our previous study(4), amylase clearances in man with normal urine flow were determined and found to fall in the range, 1-3 ml/min. but the effect on amylase excretion and clearance of increasing urinary flow to high levels was neither determined experimentally nor could it be accurately predicted from an examination of the plot of amylase clearance *vs.* urine flow for the data available. Data on amylase clearances at high urine flows produced by water diuresis have now been obtained and are described here.

Methods. The human subjects were 10 male and 3 female medical students, ages 22-29. The subjects were instructed to drink water freely during afternoon and evening of the days prior to their individual clearance studies since the studies were carried out during a period of hot weather, July and August 1956. To produce diuresis, each subject drank 1500 ml of water in 3 equal portions at half hour intervals prior to beginning of urine collection period. At zero time, the bladders were emptied and the last 500 ml of water taken. Approximately one hour later (time accurately recorded) the bladders were again emptied and these urine specimens collected. At approximately the mid-point of the intervening hour, blood samples were drawn by venipuncture for serum amylase determinations. Both urinary and serum amylase levels were determined by Van Loon's(5) method. Clearances were calculated using the standard formula $\frac{UV}{P}$ = clearance, where U = urine concentration, P = plasma or serum concentration and V = urine flow in ml per minute. All values for urine flow, amylase clearance and amylase excretion are corrected to 1.73 square meters of body surface. The diuresis produced such dilute urine that urinary amy-

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