were not determined. Since, however, detoxification of the phenol fraction is known to occur rapidly and does not cause degenerative changes in the central nervous system, the late degenerative changes associated with triphenyl phosphite may be presumed to be caused by relatively small concentrations of phosphorus acid acting over long periods. The preferential absorption of the phosphorous acid fraction by the gray matter is of interest in this connection.

## 11663

## Effect of Pantothenic Acid on the Nutritional Achromotrichia.

KLAUS UNNA AND W. L. SAMPSON. (Introduced by H. Molitor.)
*From the Merck Institute for Therapeutic Research, Rahway, N. J.*

Greying of the fur was first observed by Morgan, Cook and Davison[1] and Lunde and Kringstad[2] in black rats maintained on diets deficient in the vitamin B complex. Addition of preparations containing "filtrate factor",[3] of liver extracts containing pantothenic acid[4] or of brewers' yeast[2] have been reported to prevent the development of greying or to restore the black pigmentation of the hair.

In our laboratory, greying of the hair was obtained in black and piebald rats on a diet consisting of casein, vitamin free, 18%; sucrose, 67%; butter fat, 9%; salt mixture, 4%; cod liver oil, 2%, and supplemented with thiamin, riboflavin, nicotinamide and vitamin $B_6$. On this diet greying of the fur developed in approximately 80% of the animals within 4 weeks. At this time, the animals reached stationary weights and showed, in addition to the grey symmetrical patterns of the fur, signs characteristic of pantothenic acid deficiency in rats,[5] namely, thinning of the fur, generalized scaly dermatitis, inflammation of the nasal mucosa, blood-caked whiskers, and hemorrhages in various organs, particularly in the adrenal cortex. The addition of graded doses of pantothenic acid (calcium pantothenate, Merck) to the diet demonstrated that a daily supplement of 80 or 100 $\mu$g of calcium pantothenate, *e. g.*, doses

1 Morgan, A. F., Cook, B. B., and Davison, H. G., *J. Nutr.*, 1938, **15**, 27.
2 Lunde, G., and Kringstad, H., *Z. f. physiol. Chem.*, 1939, **257**, 201.
3 Morgan, A. F., and Simms, H. D., *J. Nutr.*, 1940, **19**, 233.
4 György, P., Poling, C. E., and Subbarow, Y., *J. Biol. Chem.*, 1940, **132**, 789.
5 Unna, K., *J. Nutr.*, 1940, in press.

representing the daily requirement for pantothenic acid in rats,[5] prevented, besides the above mentioned deficiency symptoms, the greying of the fur, whereas daily doses of 5, 10, or 20 μg were insufficient to prevent the greying, and 40 μg gave inconsistent results.

Comparable results were obtained with various concentrates from rice bran and liver. Although the addition of these concentrates caused better growth responses than pantothenic acid alone, their efficacy in preventing the greying of the hair was found to parallel closely their content of pantothenic acid as measured by the bacteriological assay.[6] This was shown by the addition of 10% rice bran, containing 30 μg of pantothenic acid per gram, to the diet. Assuming a food consumption of 5 to 10 g per rat, these animals received 15 to 30 μg of pantothenic acid daily. Greying of the fur as obtained under these conditions was comparable with that of the rats receiving 20 μg of calcium pantothenate daily. However, the growth rate of the rats receiving the rice bran was markedly superior, the difference being probably due to the presence of unknown growth factors in rice bran. Furthermore, the addition of 50 μg of calcium pantothenate to the diet containing 10% rice bran prevented the greying of the fur.

Calcium pantothenate, administered daily in doses of 100 μg to rats which had been maintained on the deficient diet for 6 to 10 weeks, produced a striking growth stimulation. The external deficiency symptoms and the grey color of the hair showed a somewhat delayed response. Simultaneously with an improvement of the general condition of the fur and skin, black hair started to grow. The grey patterns disappeared gradually within 3 to 5 weeks. By this time, the smoothness and the luster of the coat were restored together with the black pigmentation. The persistence of some scattered grey hairs in some of the animals gave them a "pepper and salt" appearance. With rice bran or liver concentrates similar curative effects were obtained on grey haired rats. Since the administration of large amounts of liver extracts sometimes also restored the black color of the fur only to the "pepper and salt" stage, it is felt that this might be due rather to a genetic factor in our strain than to a failure of pantothenic acid.

The results demonstrate the relation between the greying of the hair and the dietary deficiency in pantothenic acid. Furthermore, they give evidence of both the preventive and curative effect of calcium pantothenate as compared with that of concentrates of rice bran or liver. These experiments are not concerned with the pos-

[6] Snell, E. E., Strong, F. M., and Peterson, W. H., *Biochem. J.*, 1937, **31**, 1789.

sibility of preventing greying of hair by substances other than vitamins. Studies of this nature on black rats of a pure bred strain are in progress.

## 11664 P

## Toxicity of Pantothenic Acid.

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Pantothenic acid, first discovered as a growth factor for yeast,[1] has been established as a member of the vitamin B complex.[2] The vitamin was recently identified[3, 4] as *N*-($\alpha$,$\gamma$-dihydroxy-$\beta$, $\beta$-dimethylbutyryl)-$\beta'$-alanine and has been synthesized.[5]

In continuation of our studies of the toxicology of the vitamins of the B complex,[6, 7] the following investigation of the toxicity of pantothenic acid was carried out on animals maintained on completely adequate diets. Synthetic dextrorotatory calcium pantothenate was used in all experiments. Calcium pantothenate is readily water-soluble and almost neutral in reaction, the pH of a 10% solution being approximately 8.

Local effects of calcium pantothenate were studied by instillation into the eye and by subcutaneous injection. Instillation of 0.5 cc of a 10% solution into the conjunctival sac of 3 rabbits did not produce any irritation. Likewise no irritation, inflammation or abscess formation was observed in 4 rabbits following the subcutaneous injection of 1.0 cc of a 10% solution. The infiltration of the subcutaneous tissues subsided about as rapidly as following a control injection of 10 cc of saline.

Acute toxicity was studied in mice, rats, dogs, and monkeys. In mice and rats the L.D. 50 was determined following oral, subcu-

1 Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D., *J. Am. Chem. Soc.*, 1933, **55**, 2912.

2 Williams, R. J., *Science*, 1939, **89**, 486.

3 Williams, R. J., and Major, R. T., *Science*, 1940, **91**, 246.

4 Stiller, E. T., Keresztesy, J. C., and Finkelstein, J., *J. Am. Chem. Soc.*, 1940, **62**, 1779.

5 Stiller, E. T., Harris, S. A., Finkelstein, J., Keresztesy, J. C., and Folkers, K., *J. Am. Chem. Soc.*, 1940, **62**, 1785.

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

7 Unna, K., and Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 116.