

demonstrated that this organism is capable of inciting fibrous tissue proliferation in the perilobular zones in the livers of rabbits. In this connection, furthermore, it is noteworthy that the animals used by Rich and Hamilton were infested with coccidia, and that one of their control animals, which took the diet containing brewers' yeast, but poorly, developed cirrhosis. In agreement with the above is the recent work of Spellberg and Keeton,¹⁰ who reported the development of hepatic cirrhosis in a rabbit on a seemingly adequate diet which contained B-complex as found in cereal foods, and also in a guinea pig on a somewhat similar diet but supplemented with desiccated brewers' yeast.

Summary. Adult male albino rats administered a vitamin B-complex deficient diet over a period of from 6 to 13 weeks failed to develop histologic evidence of hepatic cirrhosis, in spite of the fact that they did develop the usual signs of B-complex deficiency. Data as to food and water intake as well as weight changes are presented.

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Effect of Pantothenic Acid Alone and in Natural Products on Nutritional Achromotrichia in Rats.

D. V. FROST, RUTH C. MOORE AND F. PEIRCE DANN. (Introduced by C. Nielsen.)

From the Abbott Laboratories, North Chicago, Illinois.

Within the last year much attention has been centered around the rôle of pantothenic acid¹⁻⁴ in prevention and cure of nutritional achromotrichia. That pantothenic acid can be identified *in toto* with the anti-graying potency of natural products, such as rice bran, liver and yeast, has been claimed by certain workers^{1, 3} and denied by others.^{2, 4, 5} Recently György⁶ has named biotin (vitamin H) as an additional factor in normal pigmentation, but the evidence presented was not entirely conclusive. Results of work in our laboratory

¹⁰ Spellberg, M. A., and Keeton, R. W., *Am. J. Med. Sc.*, 1940, **200**, 688.

¹ György, P., Poling, C. E., and Subbarow, Y., *J. Biol. Chem.*, 1940, **132**, 789.

² Dimick, M. K., and Lepp, A., *J. Nutrition*, 1940, **20**, 413.

³ Unna, K., and Sampson, W. L., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45**, 309.

⁴ Williams, R. R., *Science*, 1940, **92**, 561.

⁵ Nielsen, E., Oleson, J. J., and Elvehjem, C. A., *J. Biol. Chem.*, 1940, **133**, 167.

⁶ György, P., and Poling, C. E., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45**, 773.

indicate that pantothenic acid is *not* the only factor involved. What other factor, or factors, is involved remains an interesting question.*

Careful analysis of various natural products for pantothenic acid was essential to the interpretation of their anti-graying effects and for the clear postulation of the involvement of other unknown factors. The chick pantothenic acid assay method used was a modification of the Jukes method⁷ in which the whey adsorbate component of the diet was replaced by the crystalline B-complex factors. When the typical dermatitis syndrome with incrustations around mouth and eyes developed after one week on a complete diet and 2 weeks on the deficient diet, the chicks were placed on test. A solution of d-calcium pantothenate served as a standard for each group with 7 chicks per dose level. Supplements were administered by pipette and cure of dermatitis rather than growth was used as the criterion of the activity of test materials. The chick assay method was checked in all cases by a quantitative microbiological assay using *lactobacillus casei*.[†] Correlations between the two methods were generally good, although the chick method yielded much higher results with certain products, notably brewer's yeast. This discrepancy cannot be explained satisfactorily at present.

Experiments (Table I) were designed to determine the anti-graying potency of liver and yeast extracts and to correlate this potency with the pantothenic acid content. In these experiments graying was produced in from 5 to 10 weeks in young rats on the following ration: Casein (Labco) 18; sucrose, 73; salts O-M, 4; corn oil, 3; cod liver oil, 2. The B-complex factors were added to the ration in amounts calculated to provide the following amounts daily: thiamin, 20 μ g; riboflavin, 40 μ g; pyridoxine, 20 μ g; nicotinic acid, 120 μ g, and choline, 5 mg. Supplements were fed for 7 weeks, and the effect on pigmentation carefully noted. In all cases the liver extracts were more effective than their equivalence of pure synthetic d-calcium pantothenate. Although, in some instances, the anti-graying potency of extracts paralleled their pantothenic acid content, certain samples, notably liver fraction and brewer's yeast, had much higher anti-graying potency than the relative pantothenic acid content warranted. The animals which developed the gray hair syndrome in 35 days did not cure any more rapidly than those which had taken 60 to 70 days to deplete.

* Ansbacher, S. (*Science*, 1941, **93**, 164), named p-aminobenzoic acid as a chromotrichia factor for rats after this paper had been prepared.

⁷ Jukes, T. H., *J. Biol. Chem.*, 1937, **117**, 11.

[†] Method of Prof. F. M. Strong conducted by Eleanor Roedger.

A butanol extract of autolyzed liver proved to be an excellent source of the anti-graying factors supplementary to pantothenic acid. This fraction fed at a level calculated to supply only 15 μg of pantothenic acid per day failed to cure graying. When 35 μg of synthetic d-calcium pantothenate was added to this therapy, the graying was clearly alleviated though not completely cured in all rats.

Synthetic d-calcium pantothenate (200 μg) was injected daily for a period of 7 weeks into 3 piebald rats after graying had developed. At the end of this period there appeared to be no difference between the injected rats and the controls, which had been fed an equal amount of calcium pantothenate. All of the rats were about equally gray at the end of the experimental period and had gained weight constantly throughout the experiment.

Concurrently with the fore-mentioned work we attempted to determine the effect of varying levels of d-calcium pantothenate on growth and incidence of graying in young black or piebald rats on a highly purified diet. The composition of the diet was as follows: Casein (Labco-refluxed and washed with 95% 3A ethyl alcohol), 74; corn oil, 3; salts, 4; haliver oil, 1; thiamin, 0.4 mg; riboflavin,

TABLE I.
Pantothenic Acid of Natural Products Versus Synthetic d-Calcium Pantothenate in the Cure of Achromotrichia.

Sample	Daily dose, mg	Pantothenic acid in daily dose, μg		Curative effect on gray hair syndrome*
		Chick assay	Microbiol. assay	
Liver Paste X	50	25	28	1P, 3N
" " X	100	50	55	4C
" " W	100	45	53	2C, 3P
" " A	100	40	49	2C, 2P, 1N
" " A	150	60	74	1C, 2P, 1N
" Powder W	100	60	34	3P, 1N
" " W	150	90	51	4C
" " Y	150	56	25	2C, 2P
" " Y	200	75	33	1C, 2P, 1N
95% Alcohol Sol. Liver Paste	100	50	46	4C, 1P
Brewers' Yeast Powder	250	30	4	4P, 1N
" " "	500	60	8	4C
Ca Pantothenate	.025			1P, 3N
" "	.050			2P, 3N
" "	.075			5N
" "	.100			1C, 4P
" "	.200†			1P, 2N
" "	.200‡			2P, 1N

*C—Complete cure. P—Partial cure. N—No cure.

†Oral administration.

‡Intramuscular administration.

0.4 mg; pyridoxine, 0.4 mg; nicotinamide, 20 mg; and choline, 100 mg. Black and piebald rats 21 days old were divided into 4 groups of 6 each, with 3 males and 3 females in each group. The amounts of d-calcium pantothenate supplied daily by mouth were: Group I, 15 μ g; Group II, 30 μ g; Group III, 60 μ g; and Group IV, 120 μ g. Composite growth curves for the 6 rats in each group are shown in Fig. 1. There appeared to be no difference in the susceptibility of any of these groups to graying, although the degree of adequacy of pantothenic acid is clearly reflected in the gradation of the growth curves. Several of the rats were finally fed higher levels of d-calcium pantothenate, *i. e.*, up to 200 μ g per day, without apparent benefit. Unna⁸ has suggested 80 μ g of d-calcium pantothenate per day as the minimum requirement for optimal growth and this is in line with our findings.

Contrary to the reports of Unna⁹ and Jukes¹⁰ we did not observe successful matings on the last mentioned highly synthetic diet with added calcium pantothenate. In each case the female apparently destroyed the male within 4 days after they were caged together. Prolonged bleeding from small paw injuries was noted in many

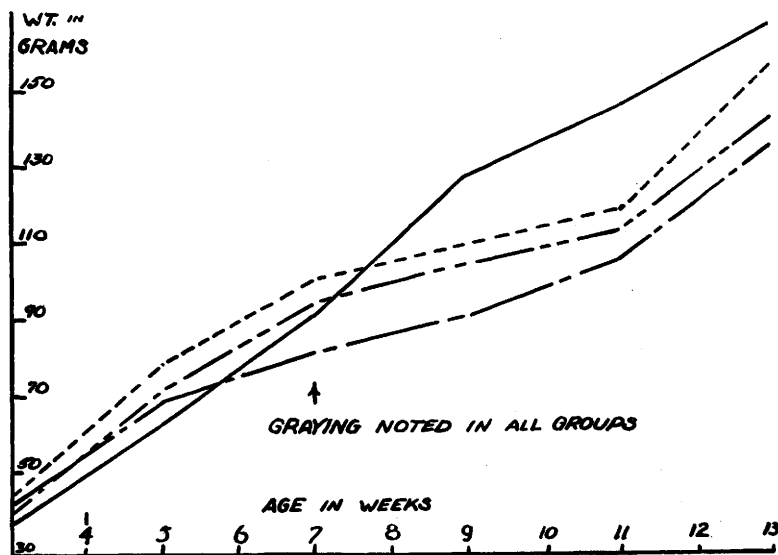


FIG. 1.

Effect of graded daily doses of synthetic d-calcium pantothenate on rat growth and complete inefficacy to prevent graying.

⁸ Unna, K., *J. Nutrition*, 1940, **20**, 565.

⁹ Unna, K., *Am. J. Med. Sci.*, 1940, **200**, 848.

¹⁰ Jukes, T. H., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45**, 625.

cases. Jukes¹⁰ noted a like condition in rats on similar rations and suggested that a vitamin K deficiency might exist.

Conclusion. The effect of pantothenic acid in cure of nutritional achromotrichia is difficult to assess at present because other factors are clearly involved. Certain liver extracts providing as low as 40 μ g of pantothenic acid per day caused complete remission, while the effect of synthetic d-calcium pantothenate was negligible at best. The addition of d-calcium pantothenate to extracts low in this factor, however, increased their anti-graying potency. Age or weight increase of animals did not seem to influence the curative effect of supplements for graying.

Although rats were raised to maturity on highly synthetic diets supplying adequate d-calcium pantothenate, successful matings were not observed.

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Hyperplasia and Hypertrophy of the Mucosa of Larger Biliary Ducts in Mice Receiving Estrogens.*

W. U. GARDNER, EDGAR ALLEN AND G. M. SMITH.

From the Department of Anatomy, Yale University School of Medicine.

During the course of observations on mice which had received injections of various estrogens for prolonged periods an enlargement of the bile ducts was observed in many animals. In untreated mice the bile ducts, cystic duct, common duct and its branches, were small thread-like structures. Occasionally a slight cystic distension of the common bile duct was apparent at autopsy. The walls were delicate and when slightly distended almost transparent. Histologically the ducts had a thin and irregular muscularis, and a well-developed mucosa. The tall columnar epithelium was moderately folded on the loose underlying tissue of the lamina propria, and a few small glands extended to the serosa, especially in the common bile duct (Fig. 3).

The bile ducts of many mice which had received estrogens were grossly thickened to several times the size of the ducts of the untreated (Fig. 1). They are also rather rigid, white and somewhat

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