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Growth and Graying of Rats with Total "Filtrate Factor" and with Pantothenic Acid.*

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Rats maintained on a purified ration supplemented with thiamin, riboflavin and pyridoxin fail to grow and solid colored and hooded rats exhibit a characteristic graying of the hair (achromotrichia).

A number of source materials (liver, rice bran and cane molasses) contain a factor or factors that remain in the filtrate after adsorption of extracts of these materials on fuller's earth. The unadsorbed fraction has been termed "factor II" or the "filtrate factor".

Most investigators are agreed that pantothenic acid has a marked stimulating effect on the growth of rats deficient in the so-called "filtrate factor"; however, some workers have reported a complete cure of or prevention of graying and others have reported no effect on pigmentation.

Experimental. Male rats were placed on diet 820† at weaning (21 days) and received in addition 15 μg of thiamin 6 times weekly. At the end of 4 weeks all animals had attained a constant weight. Six animals from different litters were used in each assay and each litter was represented by one control. Hooded or solid colored rats were employed. All animals were then given 6 times weekly in addition to the thiamin, 20 μg of riboflavin and 15 μg of pyridoxin. The test substance was administered with the supplements.

Calcium pantothenate was fed at 3 levels: namely, at 40, 50 and 100 μg daily (except Sunday) (Table I). The gain in weight above the controls for the rats receiving the 3 levels of Ca pantothenate would indicate that 100 μg of Ca pantothenate produces little augmentation in growth above that obtained with 40 or 50 μg. On

* Aided by grants from the Research Board and the Department of Agriculture of the University of California, Rockefeller Foundation and Merck & Co., Rahway, New Jersey. Assistance was rendered by the Works Projects Administration, Official Project No. 65-1-08-62, Unit A-5.

The following materials were generously contributed: thiamin, pyridoxin and Ca pantothenate, Merck & Co.; riboflavin, Hoffman La Roche Co.; 92% liver extract, Eli Lilly & Co.; molasses, Pacific Molasses Company.

† Diet 820: extracted casein[1] 24, salts (McCollum, No. 185) 4, Crisco 8, cod liver oil 2, sucrose 62.

[1] Halliday, N., and Evans, H. M., *J. Nutr.*, 1937, **13**, 657.

the other hand, the animals receiving the lower levels were completely gray while those receiving 100 μg were stippled—the so-called "salt and pepper" effect‡—a finding in agreement with Unna and Sampson[2] and with Dimick and Lepp.[3] Unna[4, 5] found that the daily requirement of pantothenic acid for growth was in the neighborhood of 80-100 μg.

The equivalent of 5 g of liver in the form of a liver filtrate (from which the thiamin, riboflavin and pyridoxin had been removed by

TABLE I.
"Filtrate Factor" Activities of Ca Pantothenate and Other Preparations.

Preparation	Quantity fed 6 × weekly,	Gain in wt above controls 60 days	Graying of rats
	μg	g	
Ca pantothenate	40	57	Yes
	50	50	"
	100	62	Stippled
Liver filtrate	equivalent of 5 g liver	95	No
Liver filtrate + Ca pantothenate	equiv. of 5 g liver + 100 μg Ca pantothenate	92	"
Ether extr. from cane molasses	equiv. of 3 g molasses	64	"
Chloroform extr. from ether extr.	equiv. of 3 g molasses	4	Yes
Inositol	1 mg	13	"
Ca pantothenate	100μg	67	Stippled
Inositol + Ca pantothenate	1 mg inositol + 100 μg Ca pantothenate	69	"
Choline hydrochloride	1 mg	0	Yes
Choline hydrochloride + Ca pantothenate	1 mg choline hydrochloride + 100 μg Ca pantothenate	69	Stippled

‡ Two gray rats were given 100 μg of Ca pantothenate daily for 4 months. After a period of 2 months a "salt and pepper" appearance was noted which persisted despite continued feeding of the Ca pantothenate.

2 Unna, K., and Sampson, W. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 309.

3 Dimick, M. K., and Lepp, A., *J. Nutr.*, 1940, **20**, 413.

4 Unna, K., *J. Nutr.*, 1940, **20**, 565.

5 Unna, K., *Am. J. Med. Sci.*, 1940, **200**, 848 (Soc. Proc.).

multiple adsorptions on fuller's earth) stimulated growth to a greater extent than did 100 μg of Ca pantothenate. The rats receiving the liver filtrate were completely protected against graying.

We have shown previously[6, 7] that a preparation obtained from molasses by continuous extraction with ether§ from acid solution both stimulated growth and prevented graying. The residue from this extraction stimulated growth but did not prevent graying. We have frequently observed graying (stippling or definite patterns) in animals receiving a diet containing 10% yeast. Such rats often show the graying during the period of rapid growth but gradually darken after that time. Rats weighing between 250 and 300 g may still show an outline of this pattern.

It would, therefore, appear that the requirement for pantothenic acid for maintaining normal color of the fur is greater than for growth or that some factor or balance of factors, in addition to pantothenic acid is required. Biotin[9] and p-aminobenzoic acid[10, 11] have been suggested as anti-achromotrichia factors.

Woolley[12] has shown that inositol both stimulates growth and prevents alopecia in the mouse. In a preliminary experiment 1 mg of inositol daily was fed to rats both with and without calcium pantothenate (100 μg) and little effect was noted. (Table.) It is, however, of interest to note that 4 of the 6 filtrate factor free controls died while all animals receiving inositol survived, and although in poor condition the rate of growth was greater than for the remaining 2 controls. Six rats receiving Ca pantothenate grew at the same rate as did 6 rats receiving inositol in addition to the Ca pantothenate and the condition of the fur was essentially the same as for the Ca pantothenate alone.

Diet 820 is sufficiently high in its casein content (24%) to protect against choline deficiency; nor did the addition of choline to

[6] Mohammad, A., Emerson, O. H., Emerson, G. A., and Evans, H. M., *Science*, 1939, **90**, 377.

[7] Mohammad, A., Emerson, O. H., Emerson, G. A., and Evans, H. M., *J. Biol. Chem.*, 1940, **133**, 17.

§ A chloroform extract prepared from molasses according to the method employed by Nielsen and coworkers[8] in the isolation of a crystalline material from liver which prevented graying, neither stimulated growth nor prevented graying. The ether extract from which the chloroform extract was prepared was active in both respects.

[8] Nielsen, E., Oleson, J. J., and Elvehjem, C. A., *J. Biol. Chem.*, 1940, **133**, 637.

[9] György, P., and Poling, C. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 773.

[10] Ansbacher, S., *Science*, 1941, **93**, 164.

[11] Martin, G. J., and Ansbacher, S., *J. Biol. Chem.*, 1941, **138**, 441.

[12] Woolley, D. W., *Science*, 1940, **92**, 384.

calcium pantothenate further improve the filtrate factor deficiency picture (Table).

Summary. Calcium pantothenate stimulates growth and prevents pattern graying (but not stippling) in filtrate factor deficient rats. The requirement for growth is less than for the prevention of pattern graying. Black rats receiving Ca pantothenate either prophylactically or curatively have stippled fur.

A liver filtrate stimulates growth to a greater extent than does Ca pantothenate and completely protects against graying. It would thus appear that pantothenic acid does not completely replace liver filtrate.

A concentrate prepared by the continuous extraction of cane molasses with ether both stimulated growth and prevented graying while a chloroform extract prepared from it was without activity.

Inositol added to Ca pantothenate does not significantly improve the results obtained with calcium pantothenate alone.

Rats maintained on a 24% casein diet apparently have no need for choline.

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Course and Chemotherapy of Experimental Relapsing Fever in the Chinese Hamster (*Cricetulus griseus.*)

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Many attempts have been made to develop in laboratory animals experimental relapsing fever of sufficient intensity and duration to permit immunologic and therapeutic studies. While monkeys and rodents are susceptible, different strains of relapsing fever spirochetes cause considerable variation in the reaction of the host. Most spirochetes produce infections of short duration in rats, mice and guinea pigs. One exception is *S. sogdianum,* a rare spirochete, which produces in guinea pigs a disease characterized by 2 to 4 relapses.[1]

While the treatment of relapsing fever usually is successful with the arsphenamines many strains of spirochetes have not responded

[1] Sautet, J., *Marseille-Méd.*, 1937, **74**, 273; from *Trop. Dis. Bull.*, 1938, **35**, 499.