A COMPARISON OF VARIOUS DIETS IN THE STUDY OF ACHROMOTRICHIA AND GROWTH OF RATS

by

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INTRODUCTION

The description of the syndromes resulting from the deficiencies of the individual vitamins in the diets of rats make up a very important part of the study of these dietary factors. However, variations in observations, basal rations, methods of feeding and care, and uncontrollable factors such as the differences in the vitamin requirements of different strains of rats have led to considerable confusion in the literature.

In the entire field of vitamin deficiencies affecting the condition of the skin and the quality and color of the hair, several factors including panthothenic acid, pyridoxine, certain of the fatty acids, and p-aminobenzoic acid appear to be involved. The interrelationships existing between these factors and the influence of other vitamins and feed ingredients have not been adequately studied.

Grey hair was observed in rats fed on a bread and whole milk diet as early as 1923 (17). It was later shown by Gorter (14) that this type of greyness was due to a deficiency of copper in the diet. In 1938, Morgan et al (30) reported a greying of black rats in which copper had no curative effect.

An attempt to find the specific factors involved in the cure of this achromotrichia (grey hair) has opened a new field for investigation. Several conflicting reports regarding the factors involved have already made their appearance. Henderson, et al (19) have reported that pantothenic acid will prevent or cure grey hair in black rats. Ansbacher (1), on the other hand, has found that in black rats fed a liberal quantity of this vitamin, an achromotrichia develops which can be cured by the administration of p-aminobenzoic acid.

The diets used by Henderson, et al and Ansbacher differed in several respects in quality and quantity of fat and vitamins. If the differences in the results reported had a real nutritional basis, then it was probably due to the differences in quality and quantity of fat and vitamins fed because otherwise the diets were practically the same.

The purpose of this work was to study the effect of interchanging some of the components of these two diets on growth and achromotrichia with black rats as the experimental subjects.

REVIEW OF LITERATURE

As early as 1930, Norris and Ringrose (33) described a pellagra-like syndrome in chicks. By 1936, thiamin and riboflavin were available in crystalline form and the symptoms resulting from their absence in the diet had been fairly well characterized. It was known that they could be readily removed from aqueous rice bran or liver extracts by a relatively small amount of fuller's earth. Lepkovsky, Jukes, and Krause (22) found that the filtrate from the above absorption could be treated with more fuller's earth to absorb a Factor I which proved to be vitamin B_6 or pyridoxine. The filtrate still contained a Factor II, or the filtrate factor. The filtrate factor prevented or cured the chick dermatitis, and both factors were essential for rat growth.

In 1938, Williams et al (48) separated pantothenic acid, a yeast growth factor, from liver. Jukes (20) and Woolley, Waisman, and Elvehjem (52), working with samples furnished by Williams, found it to be identical with the filtrate (chick antidermatitis) factor. Unna (44), in the following year, described the pantothenic acid deficiency symptoms of the rat. He found 80 γ per rat per day to be the approximate maintenance dose for optimal growth.

In 1923, Hartwell (17) reported a greying in rats during hot weather on a bread and whole milk diet. He attributed this greying to the lack of tryptophane and tyrosine for melanin or black pigment formation. But Keil and Nelson (21) showed copper to be the curative factor for greying in rats on a whole milk diet. Gorter (13) produced a yellowish depigmentation on a synthetic diet. After trying many vitamins and minerals, copper was again found to be the only active principle for prevention or cure (14).

In 1938, Lunde and Kringstad (23) and Morgan, Cook, and Davison (30) reported greying in piebald and black rats respectively. Morgan et al reported copper to have no curative effect. Lunde and Kringstad (24) concluded, the following year, that the growth-promoting filtrate factor was not identical with the anti-greying filtrate factor. Morgan and Simms (31) stated that injections of commercial adrenal cortex and thyroid extracts cured the greying in rats slowly but did not restore growth. They also found that dogs and silver foxes became grey on the filtrate factor free diet. Oleson, Elvehjem, and Hart (34) reported that pantothenic acid concentrates were ineffective in preventing the greying now becoming known as experimental nutritional achromotrichia.

Free (11) settled the question of the existence of more than one type of achromotrichia. He showed conclusively that there are at least two types of greyness in rats: that resulting from a deficiency of iron, copper, and manganese and accompanied by nutritional anemia, and that resulting on a synthetic diet in which the hemoglobin level is normal, and curable with rice bran but not with rice bran ash.

The reports on the inefficacy of pantothenic acid and the belief that the filtrate factor contained vitamins other than pantothenic acid which cured nutritional achromotrichia were made by several workers before pure pantothenic acid was available and before the pantothenic acid requirement of the rat was known. György and Poling (15) found synthetic pantothenic acid to have a curative action. Unna, Richards, and Sampson (45) fed black and piebald rats on a diet adequate except for the absence of pantothenic acid. They observed greying of the fur in three to seven weeks along with retardation of growth, the appearance of scant coarse fur, inflammation of the nose, "blood-caked" whiskers, and adrenal hemmorrhages. Development of the achromotrichia and other lesions in normal rats was prevented by the addition of 100 γ of Ca pantothenate per rat per day. It restored, within three to four weeks, the black pigmentation to the rats which had become grey. They found the ability of liver and rice bran to cure achromotrichia to parallel their pantothenic acid content although they exerted a growth-promoting effect superior to that of pantothenic acid. Biotin, inositol, and p-aminobenzoic acid were found to be ineffective, giving no differences in greying, growth rate, or the presence of scattered grey hairs persisting after addition of pantothenic acid to the diet. A 68 percent level of sucrose and cerelose were used interchangeably in their diets as were eight percent levels of hydrogenated vegetable oil (Crisco) and butterfat without any apparent differences in results. Henderson et al (19) studied only pantothenic acid and p-aminobenzoic acid with similar results.

Mushett and Unna (32) found the daily administration of adrenal cortical extract, desoxycorticosterone acetate, thyroid, and anterior pituitary extract to have no preventive or curative action on greying of hair or occurrence of adrenal hemorrhages in rats on pantothenic acid deficient diets. McElroy et al (26) found the red deposit around the nose and on the whiskers of pantothenic acid deficient rats to be coproporhyrin from the Harderian gland and not blood as it had been previously believed.

Not all workers have been able to maintain complete fur color through the use of pure Ca pantothenate. Williams (47) reported the failure of either pantothenic acid concentrate or synthetic Ca pantothenate to prevent or cure rat achromotrichia. György and Poling (16) found biotin to be an additional factor. Emerson and Evans (9) found Ca pantothenate to prevent pattern greying but not salt and pepper greying which they called stippling. They reported a liver filtrate to protect completely against greying.

In 1940, Woods (49) found p-aminobenzoic acid to have a high activity in antagonizing sulfanilamide. Rubbo and Gillespie (39) isolated N-benzoyl-p-aminobenzoic acid from brewers' yeast and found it to be a growth factor for bacteria. Ansbacher (1) reported p-aminobenzoic acid to be a vitamin the following year. He fed 500 γ each of Ca pantothenate, inositol, and nicotinic acid, 3 mg of choline chloride, and 40 γ each of thiamin, pyridoxine, and riboflavin in $\frac{1}{2}$ ml of 20 percent ethanol solution to each rat daily in addition to a synthetic basal diet. On the appearance of a definite greying, a second daily supplement of 1 ml of 20 percent ethanol solution containing 3 mg of the p-aminobenzoic acid per milliliter was fed. A bluish discolorization of the skin typical of the first sign of growth of normally pigmented hair appeared in two to three weeks. Black hair appeared within a month.

Martin et al (29) reported p-aminobenzoic acid to have a modifying effect on melanin formation and pantothenic acid to have none. It was also shown that p-aminobenzoic acid prevented greying in mice on a high pantothenic acid diet (28). Sure (43) showed it apparently to be a dietary essential for lactation and reproduction in the rat. Sieve (40) reported it as well as a vitamin B complex preparation to be active in curing achromotrichia in humans. Martin (27) reported that p-aminobenzoic acid deficiency in rats is characterized by slight greying. He further concluded that the ratio of Ca pantothenate to p-aminobenzoic acid was the important factor, and that a ratio favoring the latter resulted in greying. He offered the explanation that there is a destruction of pantothenic acid by microorganisms which are stimulated in their growth by p-aminobenzoic acid. Ansbacher, Wisansky, and Martin (2) showed that the acid probably has a protecting or sparing action on certain hormones. Richards (37) found it to have a low acute and chronic toxicity.

There have also been reports in which p-aminobenzoic acid has been shown not to be effective in connection with achromotrichia and alopecia. The studies of Unna et al and Henderson et al have already been cited. Emerson (8) failed to get any curative response with p-aminobenzoic acid on rats previously greyed on a pantothenic acid deficient diet. Woolley (51) found it ineffective in the treatment of alopecia in mice. Richter and Clisby (38) produced achromotrichia in rats by administering the drug phenylthiocarbamide, showing that a positive factor, a poison, may cause greying as well as a deficiency of an essential factor.

Inositol is one of the more recently reported members of the vitamin B complex. In 1941, Pacvek and Baum (35) reported inositol to be a growth-promoting factor for the rat and to prevent the symptom known as "spectacle eye". Woolley (50) demonstrated that the mouse required inositol for normal growth and the prevention of alopecia. Pantothenic acid was also found to be an anti-alopecia factor, and it was also noted that many pantothenic acid deficient mice were unable to open their eyes and showed a paralysis of the hind legs. Hegsted et al (18) stated that inositol has a definite growth-promoting action in the chick although no other pathological symptoms had been produced by its lack.

In 1937, Elvehjem et al (7) found nicotinic acid and nicotinamide highly effective in curing canine black tongue. The following year, Spies, Bean, and Stone (41) reported it beneficial to both the physical and mental symptoms of pellagra. Madison, Miller, and Keith (25) cured swine pellagra with nicotinic acid. Dann and Kohn (5) reported that rats were able to synthesize nicotinic acid, and that the rate of growth was not increased by adding it to the diet. Their findings indicated that nicotinic acid is not a vitamin for the rat. Stekol (42) recently found one percent niacin (nicotinic acid) to inhibit growth in young male rats fed a casein low diet. Methionine or cystine promptly alleviated the inhibition, but neither choline nor glycine were effective. No niacin inhibition was observed in the female. His observations also suggested that niacin was converted principally into trigonelline in the male but not in the female, and that methionine or its labile methyl group is involved.

In 1935, Best and Huntsman (3) proposed that choline be considered a dietary essential in the rat. Griffith (12) stated that the daily requirement of choline varies with the dietary methionine, cystine, betaine, and cholesterol as well as with the adequacy of the ration for optimum growth. Choline-like action of methionine and betaine appears satisfactorily explained on the basis of transfer of methyl groups by the process of transmethylation proposed by du Vigneaud. Deuel et al (6) stated that rats previously on a high butterfat diet supplemented with choline did not accumulate fat in the liver. Engel and Salmon (10) produced fatal toxicity and described the external and microscopic symptoms of rats on a choline deficient diet. The symptoms were prevented by feeding 20 to 30 mg of choline chloride per rat daily. Emerson (8) reported that rats maintained on a 24 percent casein diet apparently do not need choline.

EXPERIMENTAL PROCEDURE

The purpose of this work was to study the growth and achromotrichia produced in black rats on the Henderson, et al diet (19), the Ansbacher diet (1) and six other diets prepared by interchanging the different fats and vitamins of the two diets.

Eight nearly equal groups of five 24-day-old black rats each were used on the experimental diets. Each group contained three males and two females[1]. Ten rats, three males and seven females, were fed the stock fox-chow diet and used as positive controls. All the rats were kept in individual cages set on coarse woven-wire trays. They were given food and water ad libitum, and their weights and gains in weight were recorded each week.

The eight experimental groups were designated alphabetically, Groups A to H, and they received Diets A to H respectively. These rats were numbered consecutively one to 40 beginning with the first rat in Group A and ending

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[1] The first group contained two males and three females.

with the last rat in Group E. The controls were numbered from 40 to 50.

The experimental diets were prepared with the compositions shown in Table 1. Diet A was the Henderson et al diet with sucrose replaced by cerelose. Diet B was the Ansbacher diet with agar replaced by cellu flour, a different salt mixture, and with the vitamins fed by mixing into the feed instead of feeding separately in a 20 percent ethanol solution.

The six other diets were made up by interchanging the cellu flour, soybean oil, Crisco, and cod liver oil of the Ansbacher diet for the corn oil and Natola of the Henderson, et al diet[2], and by adding different vitamin mixtures to the diets. In this way it was hoped to find a "hidden" factor or hidden factors which were responsible for the differences in the results obtained by Ansbacher and the Wisconsin workers.

The salt mixture used is shown in Table 2 and is based on that of Phillips and Hart (36) with $MnSO_4$ increased as recommended by Conger and Elvehjem (4).

The casein used was extracted with alcohol. The Natola which was used as a source of vitamins A and D,

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[2] The cerelose level was either raised or lowered to make up for the difference in weight of corn oil and Ansbacher's fat mixture plus cellu flour.

Table 1. Compositions of the experimental diets used.

Ingredients	Rat Groups A Nos. 1-5	B Nos. 6-10	C Nos. 11-15	D Nos. 16-20	E Nos. 21-25	F Nos. 26-30	G Nos. 31-35	H Nos. 36-40
Cerelose	730 g	700 g	700 g	730 g	700 g	730 g	730 g	700 g
Casein	180	180	180	180	180	180	180	180
Salt Mixture	40	40	40	40	40	40	40	40
Corn oil[3]	50			50		50	50	
Cellu flour		20	20		20			20
Soybean oil		20	20		20			20
Crisco		20	20		20			20
Cod liver oil		20	20		20			20
Choline chloride	1	0.3	1	0.3	1	1		0.3

Table 1 (cont.)

Thiamin	2 mg	4 mg	2 mg	4 mg	4 mg	2 mg	2 mg	4 mg
Pyridoxine	2	4	2	4	4	2	2	4
Riboflavin	3	4	3	4	4	3	3	4
Nicotinic acid	2.5	50	2.5	50	50	50	2.5	
Inositol		50		50	50	50		
Ca pantothenate		50		50	50	50	50	50
Natola	2 drops ea.wk.			2 drops ea.wk.		2 drops ea. wk.	2 drops ea. wk.	

[3]Mazola.

Table 2. Composition of the salt mixture used.

Salts	Grams
NaCl	335
$K_2HPO_4 \cdot 3H_2O$	845
$Ca(H_2PO_4)_2 \cdot H_2O$	140
$MgSO_4 \cdot 7H_2O$	204
$CaCO_3$	630
$Fe\ (C_6H_5O_7)_2 \cdot 6H_2O$	55
KI	1.6
$MnSO_4 \cdot H_2O$	17.0
$ZnSO_4$	0.6
$CuSO_4 \cdot 5H_2O$	0.6
Total	2228.8

contained 55000 U.S.P. units of A and 5500 units of D per gram.

In order to facilitate the mixing of the feed, the salt mixture was dehydrated, and the amount used was recalculated to allow for the loss of water of hydration.

The casein and dehydrated salt mixture were ground separately in the Wiley mill and mixed with cerelose by hand. Cellu flour was mixed in similarly. The fats were mixed in by starting with a small addition of the diet to the fat and adding more gradually until the fat was mixed uniformly throughout the feed. The proper vitamins were mixed into the different diets by the same method used for the fats. The choline was ground into a small amount of ration in a mortar as a preliminary step to the above procedure.

The rats were kept on the diets shown in Table 1 for six weeks. During this time, only Groups A and C, the only groups not receiving pantothenic acid, had developed achromotrichia. Their weights were significantly lower than those of the other groups also. At the beginning of the seventh week the diets of Groups A and C were changed in an attempt to cure their achromotrichia. Diet A was supplemented by mixing p-aminobenzoic acid into the diet at a level of 300 mg per 1000 g. Group C was shifted to Diet B, the diet used by Ansbacher to produce greyness although it contained a high level of pantothenic acid.

Two weeks later, the rats in the six other groups had developed some scattered grey hairs or a slight salt and pepper greyness. A method of judging the amount of greyness in the eight groups was devised. Their growths were also studied.

It was decided to supplement some of the diets at this time in an attempt either to cure the slight salt and pepper greyness or to produce a more definite achromotrichia.

Diet H and part of Diet B were supplemented with 300 mg of p-aminobenzoic acid per 1000 g of feed. This made the supplemented Diet B almost identical in composition to the diet Ansbacher used to cure achromotrichia. The supplemented Diet B was fed to Group B, and in addition to rat No. 15 of Group C which was entirely grey and to No. 2 of Group A which was entirely grey ventrally but otherwise black (symmetrical greyness). The rest of Group C which contained rats Nos. 11 and 12 having the same type of greyness as Nos. 15 and 4 respectively, were left on the unsupplemented Diet B. If the two rats on the Diet B supplemented with p-aminobenzoic acid were cured of greyness while the two on the unsupplemented Diet B failed to respond, it would be at least an indication of the beneficial action of p-aminobenzoic acid on achromotrichia.

Group A was not responding to p-aminobenzoic acid in the absence of pantothenic acid, so the diet was resupplemented to study the importance of the pantothenic acid-p-aminobenzoic acid ratio as suggested by Martin (27). Ca pantothenate and p-aminobenzoic acid were added to 1000 g of Diet A at the levels of 10 mg and 500 mg respectively. An even lower ratio of pantothenic acid to p-aminobenzoic acid, or 10 mg Ca pantothenate and 1 g p-aminobenzoic acid were added to 1000 g of Diet E. Nicotinic acid was added to Diet F at a one percent level to see if its reported toxic effect on male rats would have any influence on their achromotrichia.

Group D was left on the unsupplemented Diet D to act as a control group. Group G was destroyed to conserve feed.

After 12 weeks, or four weeks after the above supplementing, the achromotrichia of all the groups was again studied.

RESULTS AND CONCLUSIONS

Growth

Table 3 gives the average weekly weights and average total gains of the eight groups for the first eight weeks of the experiment. The slow growth of Groups A and C was due to the absence of pantothenic acid in these diets. The sharp increase in the rate of gain of Group C beginning with the seventh week was due to the addition of pantothenic acid to the diet. The p-aminobenzoic acid added to Diet A at the same time had no effect.

A comparison of the growth and diets of the other groups gave several indications of the growth-promoting value of the fats and the vitamin mixtures which varied from diet to diet.

Table 3. Average weights and average total gains in grams for eight groups of five rats each.

Groups	Average initial weights	Average weight at end of weeks 1	2	3	4	5	6	7	8	Average total gains
A	30.8	50.0	66.9	82.2	89.6	97.4	106.9	112.1	121.3	90.5
B	31.7	49.9	72.1	96.6	116.6	141.8	165.5	181.2	201.7	169.6
C	36.6	55.7	75.1	88.6	93.6	103.8	113.3	140.8	171.0	134.4
D	32.5	53.3	73.2	99.5	125.4	150.9	175.1	188.6	208.0	175.5
E	33.1	55.2	83.6	111.1	134.0	158.9	182.0	200.5	212.8	179.7
F	33.2	52.1	73.3	96.2	119.9	145.1	168.4	185.5	199.9	166.4
G[4]	36.2	51.4	75.9	102.5	121.0	147.0	167.8	183.5	199.1	162.9
H	33.1	54.8	81.7	102.4	129.3	155.9	176.1	192.1	211.1	177.9

[4] One rat in Group G died during the second week on the experimental diets and was replaced from the positive controls.

Interchanging cellu flour, soybean oil, Crisco, and cod liver oil of the Ansbacher diet for corn oil and Natola of the Henderson, et al diet seemed to have no effect. This can be shown by comparing the average total gains of Group D, receiving corn oil, with Groups E, H, and B receiving the "Ansbacher fats". These four groups received the Ansbacher (1) levels of thiamin, pyridoxine, riboflavin, and choline while Group F received the Henderson, et al (19) levels, and Group G received the Henderson et al levels of thiamin, pyrodoxine, and riboflavin, but no choline. The results indicate that it would be safer to feed the Ansbacher levels of thiamin, pyridoxine, and riboflavin, if the optimum growth-promoting effects of these three vitamins are desired.

The fact that young rats on an 18 percent casein diet need some choline is well established, and the fact that one member of Group G died during the second week is in agreement with this. Group E received 1 g choline per 1000 g of diet while Group B on an otherwise identical diet received only 300 mg choline per 1000 g. A comparison of the weights of Groups B and E again showed that feeding the higher level, this time the Henderson et al level, would be safer.

Group H received no inositol or nicotinic acid while Groups E, D, and B on otherwise similar or identical diets received the Ansbacher levels of these two factors. The results in Table 3 show that inositol and nicotinic acid had no effect on growth.

Achromotrichia

During the fourth week on the experimental diets, male rats No. 2 and No. 11 on the pantothenic acid-free Diets A and C respectively were definitely greying. The greyness appeared evenly distributed over the entire body except for a black patch on the head. By the beginning of the fifth week, females No. 5 of Group A and No. 15 of Group C were greying in the same manner. By the sixth week, four rats in each group showed some degree of achromotrichia. Plate I shows the difference in size and greyness of rat No. 11 of Group C and rat No. 21 of Group E on a diet containing pantothenic acid. Rats No. 4 of Group A and No. 12 of Group C were just beginning to show a symmetrical greyness starting ventrally and proceeding equally up both sides of the body. The head of rat No. 12 was grey where the rats showing the more complete type of greyness had remained black. Females No. 3 of Group A and No. 14 of Group C still were quite black. Rats No. 1 of Group A and No. 15 of Group C had difficulty opening their eyes. Most of the rats exhibited rust-colored hair on the nose and neck, typical of pantothenic acid deficiency in both albino and black rats.

Diets A and C had given almost identical results although Diet A contained corn oil while Diet C contained the fats used by Ansbacher. This showed that the character

EXPLANATION OF PLATE I

Photographs of two rats showing the effect of dietary pantothenic acid on the growth and achromotrichia of black rats after six weeks on experimental diets. The rats were of the same size and color at the beginning of the experiment.

Fig. 1. Showing the totally black coat of rat No. 21 which had received Diet E containing a liberal quantity of pantothenic acid. The grey sheen appearing in the coat was due to the photography.

Fig. 2. Showing the grey coat of rat No. 11 which had received Diet C containing no pantothenic acid.

PLATE I

Fig. 1.

Fig. 2.

of the fats was not a significant factor in these diets.

The diets for Groups A and C were supplemented with p-aminobenzoic acid and pantothenic acid respectively, as previously described, at the beginning of the seventh week.

By the next week, rat No. 1, still not receiving any pantothenic acid, had developed the only typical "blood-caked" whiskers observed, and his eye condition was worse. The eye condition of rat No. 15 of Group C, had disappeared promptly upon receiving the diet containing pantothenic acid.

At the end of eight weeks, all of the rats on the diets which had contained pantothenic acid from the beginning showed some salt and pepper greyness. The control rats showed this same condition. The rats from Groups A to H were scored for greyness by the method shown in Table 4, and the individual and total scored obtained are shown in Table 5. The rats in Groups A and C had been on supplemented diets for only two weeks which had been insufficient time for any noticeable change in their greyness.

This method of scoring brought out two important conclusions. The first conclusion was that none of the diets protected the rats completely from achromotrichia; the second conclusion was that the males were more susceptible to greying on these diets than were the females.

Starting with the ninth week the supplemented diets described on page 16 were fed. After four weeks on these

Table 4. Method of scoring the degree of greyness of black rats.

Explanation of scoring
0 -- Black
1 -- Very slight salt and pepper on sides
2 -- Slight salt and pepper spreading to back
3 -- Definite salt and pepper
4 -- Symmetrical greying
5 -- Grey
6 -- Very grey

Taole 5. Greyness scored of the rats at the end of eight weeks.

Groups and rats nos.	Scores					
	Males			Females		Totals
A (1-5)	5	6	1[5]	4	6	22
B (6-10)	2	2	2	1	2	9
C (11-15)	6	4	5	1	6	22
D (16-20)	2	2	2	1	1	8
E (21-25)	2	2	2	1	1	8
F (26-30)	1	2	2	1	1	7
G (31-35)	2	2	2	1	1	8
H (36-40)	2	2	1	1	1	7

[5] Female.

diets the rats were again scored for greyness by the same method and the results are shown in Table 6.

Table 6. Greyness Score of the rats at the end of 12 weeks.

Groups and rats nos.	Scores					
	Males			Females		Totals
A (1-5)	4	4	4	4	4	20
B (6-10)	2	2	2	2	2	10
C (11-15)	3	4	3	2	2	14
D (16-20)	3	2	3	1	2	11
E (21-25)	3	3	3	2	2	13
F (26-30)	3	3	3	2	2	13
H (31-40)	3	3	2	2	2	12

A comparison of Tables 5 and 6 shows that, after six weeks on a diet containing pantothenic acid, the three greyest rats in Group C had recovered from their complete greyness and had left only the salt and pepper greyness exhibited by all the groups except Group A. A comparison of rats Nos. 15 and 4 receiving p-aminobenzoic acid with Nos. 11 and 12 receiving no p-aminobenzoic acid did not indicate that p-aminobenzoic acid has any curative action on achromotrichia. Only striking results, however, would have been at all conclusive with the limited numbers of rats used. The symmetrically grey rats Nos. 4 and 12 had not responded noticeably on either diet.

The other groups had been on their supplemented diets for only four weeks. The "blood caked" whiskers and eye condition of rat No. 1 had been cured promptly by the low pantothenic acid-high p-aminobenzoic acid diet being fed Group A. Table 6 shows that, with the exception of No. 3, the rats in Group A were blacker than they were four weeks previously. Rat No. 3 had developed the symmetrical greyness after the addition of the pantothenic acid and p-aminobenzoic acid to the diet.

Group A developed more symmetrical greyness during repigmentation than had been noticed for Group C. Another type of symmetrical greyness was observed during the repigmentation. An area extending from the hips to the tail remained grey along with the ventral part of the body after the rest of the body had become quite black. The partial recovery of the rats in Group A did not support Martin's (27) report that a low pantothenic acid-high p-aminobenzoic acid ratio produced greyness.

All other groups, with the possible exception of Group B, were definitely greyer than they had been before the change of diets at the beginning of the ninth week. Group B was receiving the diet containing p-aminobenzoic acid which Ansbacher used to cure achromotrichia. The slight differences in greyness between Group B and the other groups could not be interpreted to be due to the differences in diets.

There appear to be three different types of achromotrichia; a complete greyness prevented or cured with pantothenic acid, a symmetrical greyness prevented with adequate pantothenic acid, but found incurable in six weeks with pantothenic acid or pantothenic acid and p-aminobenzoic acid in the amounts used by Ansbacher, and a salt and pepper greyness for which no prevention or cure was found although the time left for the cure may have been too short.

Ansbacher's diet was found to produce a slight salt and pepper greying in this study. It is possible that the different results reported on similar grey-hair studies have been due to some authors noting and some neglecting this type of achromotrichia. No hidden achromotrichia factors were found.

SUMMARY

1. Pantothenic acid is an important factor in rat achromotrichia, but there are still other factors involved in the prevention or cure of this syndrome.

2. The experiment failed to show that rat achromotrichia is related to the p-aminobenzoic acid intake.

3. Male rats are more susceptible to greying than females.

4. Under the conditions of the experiment, nicotinic acid and inositol had no effect on growth.

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