

Effect of pantothenic acid on growth and blood picture in the rat

BY ANNE D. BLUNT, ELISABETH M. CHEESMAN
AND ALICE M. COPPING

*Departments of Nutrition and Physiology, Queen Elizabeth College
(University of London), Campden Hill Road, London W. 8*

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In further studies with purified diets (Copping, Crowe & Pond, 1951; Batchen, Cheesman, Copping & Trusler, 1955) the response in growth and in blood formation to graded doses of pantothenic acid has been investigated.

EXPERIMENTAL

Animals. The 108 rats used in these experiments were of the Lister Institute black-and-white stock maintained at Queen Elizabeth College. They were weaned at 23 days and taken for experiment either at weaning, weighing 40–50 g in series C₉ and C₁₂ or 7 days after weaning, weighing 55–80 g in series C₁₅. In the first series, C₉, the animals were deprived of pantothenic acid for 2 weeks and then dosed for 4 weeks. In the two subsequent series doses were given from the beginning of the tests, for 7 weeks in series C₁₂ and for 10 weeks or longer in series C₁₅. In series C₁₅ a further group of undosed rats was maintained for 7–11 weeks and then given a small dose of pantothenic acid for 4 weeks. The changes in pigmentation and condition of the fur and other signs of pantothenic-acid deprivation were noted throughout the study.

Diets. The basal diet in all experiments consisted of casein (Genatosan Ltd, Loughborough, England, vitamin-low) 20, sucrose 60, arachis oil 12, lard 3 and salt mixture 5 parts. Vitamins A and D were given as Adexolin (Glaxo Laboratories Ltd) in a dose of 120 i.u. vitamin A and 20 i.u. vitamin D weekly. Vitamin E was given as a weekly dose of 1 mg α -tocopheryl acetate in arachis oil and vitamin B₁₂ as a weekly dose of 1 μ g of Cytamen (Glaxo Laboratories Ltd) from an Agla micrometer syringe.

The B-vitamins were given as a solution containing in a daily dose of 1 ml.:

Thiamine	10 μ g	p-Aminobenzoic acid	0.5 mg	Pyridoxine	10 μ g
Nicotinic acid	0.5 mg	Choline	1.5 mg	Folic acid	2 μ g
Biotin	0.2 μ g	Riboflavin	40 μ g	Inositol	0.5 mg

Pantothenic acid was given separately as a solution of Ca D-pantothenate from a dropping pipette calibrated to deliver doses of 25, 50, 75 and 100 μ g.

For series C₁₅, in which blood studies were made, a control group of litter-mates of the rats on the purified diet was kept on the normal stock diet of mixed grains, meat, fish, bread, milk and greenstuff.

Sampling of blood. Blood samples were taken in series C₁₅. Animals were anaesthetized by the intraperitoneal injection of a 3.3% solution of Nembutal (Abbot Laboratories Ltd, London), 0.25–1.0 ml. according to the weight of the animal.

A sample of blood from the tail was taken directly into a white-cell diluting pipette. The chest was then opened and about 1.0 ml. blood withdrawn from the right ventricle. The anticoagulant used was heparin. This blood was used for red-cell counts, haemoglobin estimations and haematocrit values.

Red-cell counts, white-cell counts and haematocrit values. The methods used were those previously described (Batchen *et al.* 1955).

Haemoglobin estimation. The method of King, Wootton, Donaldson, Sisson & Macfarlane (1948) was used with the M.R.C. grey-wedge photometer.

Red-cell diameter. Duplicate smears of heart blood were made for the measurement of mean red-cell diameters by the method of the Association of Clinical Pathologists (Bell, 1952).

RESULTS

Growth. In series C₉ with a preliminary depletion period of 2 weeks it appeared that a dose of pantothenate above 50 μ g had no effect in increasing growth response during the 4 weeks of the test. In series C₁₂ doses were given from the outset and continued for 7 weeks. Under these conditions little difference was observed with doses of above 25 μ g pantothenate. In series C₁₅ the experiment was prolonged in order to obtain maximum effects of depletion in the animals with no dose, and in Table 1, which summarizes the growth results, the responses of animals at 7 weeks and at 10 weeks are separately recorded. The growth responses for males and females are separately recorded, and although the numbers in the groups are small, the standard errors of the means in every series are small enough for the results to be considered satisfactory. In series C₉ there was a difference in response to 25 and to 50 μ g pantothenic acid by male and female rats. This difference did not appear in series C₁₅ or among female rats in series C₁₂. The rats in series C₁₅ were a week older at the beginning of the experiment and the extra days on stock diet may have prevented differences in response to graded doses of pantothenic acid. Doses greater than 50 μ g daily did not produce greater weight gains. It seemed that male rats were more sensitive than female rats in their requirement for pantothenic acid.

Recovery experiments with the seven deprived rats dosed with 25 μ g pantothenate at a time when their skin lesions and general condition indicated severe deficiency of pantothenic acid showed that the growth response to the curative dose was remarkably constant, irrespective of the length of the depletion period that preceded dosing and of the weight of the animal at the time of dosing.

Deficiency signs. In rats deprived of pantothenic acid greying of the black portions of the fur was observed, with accompanying poor condition of the fur and occasional rusty staining of tail and lower abdomen. Greying appeared after 6–9 weeks on the diet lacking in pantothenate, and little change was seen during the recovery period of 4 weeks, although the general condition of the animals improved. Some rats developed the so-called 'spectacled eye' condition. Priapism occurred in a few of the

male animals. One animal died after 13 weeks with severe greying, staining and deterioration of coat. Rats receiving 25 μg pantothenate daily showed slight greying of the black hood, even though their growth response was little less than that of rats receiving greater doses.

Table 1. *Weight increases of rats in series C₉, C₁₂ and C₁₅*

Series no.	Dose of pantothenic acid (μg)	Dosing period (weeks)	Males		Females	
			No. of rats	Mean weekly weight gain (g)	No. of rats	Mean weekly weight gain (g)
C ₉ , with 2 weeks' preliminary depletion	0	4	4	3.6	2	2.0
	25	4	4	9.6	2	6.2
	50	4	4	15.7	2	10.5
	75	4	4	16.9	2	11.9
	100	4	4	15.4	2	12.9
Standard error of the means				0.51	0.25	
C ₁₂	0	6	3	5.1	4	5.8
	25	7	4	12.2	4	11.0
	50	7	4	16.5	4	12.2
	100	7	4	17.6	4	12.6
Standard error of the means				0.58	0.59	
C ₁₅ *	0	7	4	6.8	4	5.3
	25	7	4	14.4	4	11.4
	50	7	4	14.6	4	11.6
	100	7	4	14.4	4	12.5
	Stock diet	7	4	31.6	4	19.1
Standard error of the means				0.68	0.71	
C ₁₅ *	0	10	4	4.7	4	4.0
	25	10	4	12.9	4	9.7
	50	10	4	12.2	4	9.1
	100	10	4	13.1	4	10.3
	Stock diet	10	4	26.3	4	15.2
Standard error of the means				0.45	0.54	
C ₁₅ Recovery after 7-11 weeks' depletion	25	4	4	7.9	3	7.8
Standard error of the mean				0.25	2.88	

* Weights separately recorded at 7 and at 10 weeks.

Blood. Table 2 shows the results of the blood studies on rats in series C₁₅, with the standard error of the means. Analysis of variance was used to investigate the significance of differences between means.

Red cells. The results for rats on the stock diet showed good agreement with those found in previous studies on this colony (Batchen *et al.* 1955).

No significant differences were observed between the three groups that received pantothenic acid throughout the experiment.

In the group that received no pantothenic acid the red-cell count was higher ($P < 0.01$) than in those that received pantothenic acid throughout the experiment, but the haemoglobin content of the blood and the haematocrit value were the same in all four groups. The mean corpuscular haemoglobin (M.C.H.) and the mean corpuscular volume (M.C.V.) were lower ($P < 0.01$ and $P < 0.05$ respectively) in the deprived group, but the mean corpuscular diameter (M.C.D.) did not fall, and, although there was a

slight apparent fall in the mean corpuscular haemoglobin concentration (M.C.H.C.), it was not significant.

In the group that received pantothenic acid after 7-11 weeks' deprivation (group 6), the red-cell count was similar to that in the deprived group and higher ($P < 0.01$) than that in the groups that received pantothenic acid throughout the experiment. The haemoglobin content of the blood and the haematocrit value were not significantly higher than in the other groups. The M.C.H. and M.C.V. were the same as in the groups that received pantothenic acid throughout the experiment, but the M.C.D. was greater ($P < 0.01$).

White cells. The total leucocyte count for the rats on the stock diet (group 5) was the lowest recorded in this colony, but was higher than in all other groups. No differences were found between the groups on the synthetic diets.

Table 2. *Blood values for rats in series C₁₅*

Group no.	No. of rats	Haemoglobin (g/100 ml.)	Red blood cells ($10^6/\text{mm}^3$)	Mean corpuscular haemoglobin ($\mu\mu\text{g}$)	Haematocrit (percentage of red-blood cells)	Mean corpuscular volume (μ^3)	Mean corpuscular haemoglobin concentration (%)	Mean corpuscular diameter (μ)	Total leucocytes ($10^3/\text{mm}^3$)	Lymphocytes ($10^3/\text{mm}^3$)	Poly-morphs ($10^3/\text{mm}^3$)
1	8	13.03	7.41	17.6	38.9	52.5	33.5	5.29	4.0	2.5	1.5
2	8	13.14	6.81 (7)	19.2 (7)	38.3	56.2 (7)	34.3	5.23	4.9	3.5	1.4
3	8	12.91	6.95	18.6	38.1	54.9	33.9	5.26	5.2	3.5	1.7
4	8	13.05	7.00	18.7	38.4	54.9	34.0	5.22	4.0	2.8	1.2
5	8	13.41	6.58	20.4	39.3	59.7	34.2	5.39	7.6	5.7	1.9
6	7	13.64	7.47	18.3	41.3	55.2	33.1	5.41	4.8	3.1	1.7
Standard error of the means		0.29	0.17	0.3	0.9	0.8	0.3	0.03	0.7	0.5	0.2

The figures in parentheses give numbers of rats when these differed from those in column 2. Group 1: basal diet alone. Group 2: basal diet with 25 μg pantothenic acid. Group 3: basal diet with 50 μg pantothenic acid. Group 4: basal diet with 100 μg pantothenic acid. Group 5: stock diet. Group 6: basal diet for 7-11 weeks, then basal diet with 25 μg pantothenic acid for 4 weeks.

DISCUSSION

The failure to show consistently graded growth responses to the doses of pantothenic acid used in these tests did not agree with the results reported by Bacon & Jenkins (1943), King, Strong & Cheldelin (1950) and Lih, King, Higgins, Baumann & Strong (1951). Unna (1940), in a study of the pantothenic-acid requirement of the rat, concluded that 80 μg daily was necessary for optimal growth. In our experiments the growth of the animals on purified diets was poor in comparison with that of control rats on normal stock diet, but it was similar to the growth obtained in other work involving similar diets supplemented with all known vitamins in pure form (Copping, unpublished work). In the experiments of Bacon & Jenkins (1943), which were the result of observations of deficiency signs arising in tests of B-vitamins in wheat germ (Copping, 1943*a, b*), the inclusion of wheat germ in the diet introduced unknown factors that may not be among the pure vitamins used in the present studies. The differences in growth response to pantothenic acid in Bacon & Jenkins's tests were small in comparison with the basal response to the wheat-germ supplement. All rats in their tests were male, and the statistical analysis of our results showed that a more satisfactory response to pantothenic acid was obtained with male than with female rats.

In the experiments of King *et al.* (1950) the pantothenate supplements were included in the diet, and the amounts taken varied somewhat according to the appetite of the rats. Administration of specific doses of pure pantothenate with a highly purified diet appeared to be the most satisfactory method of investigating the growth response to this vitamin, provided that male rats are used as test animals.

The changes in pigmentation of the black portions of the fur in rats deprived of pantothenic acid were similar to those reported by other observers. The unspecific skin lesions and alterations in fur condition agreed also with those reported elsewhere and reviewed by Frost (1948) and Morgan (1951). In the preliminary test there seemed to be an indication that administration of a small dose of pantothenic acid increased the extent of greying of the fur. However, it was not consistently observed in subsequent experiments. It seemed that, although a dose of 25 μg was adequate for growth, it was not always able to prevent depigmentation of fur. Since no differences in the blood picture were observed between animals receiving 25 μg pantothenic acid and 100 μg pantothenic acid daily, it would appear that 25 μg is adequate for haematopoiesis.

The only changes found in the blood picture of the rats on the deficient diet were a slight increase in the red-cell count and a slight decrease in the red-cell volume and haemoglobin content. This finding is very different from that of Daft, Kornberg, Ashburn & Sebrell (1945) and Ashburn, Daft & Faulkner (1947). They fed albino rats on a purified diet deficient in pantothenic acid for 24–95 days from weaning, with an average of 50 days on the diet. They found that in 70% of their animals blood dyscrasias developed, either as severe anaemia with a fall in red-cell count, or as granulocytopenia or as both. They did not include folic acid in their diets and attributed the granulocytopenia to folic-acid deficiency caused by the lack of pantothenic acid. They did not associate the anaemia specifically with the lack of pantothenic acid, but considered that it might be due to some factor bearing a metabolic relationship to pantothenic acid similar to that borne by folic acid. Treatment of anaemic animals with folic acid, however, showed that it was not the factor. Folic acid was included in our diet, which might account for the absence of granulocytopenia in our animals. We also included several other factors omitted from the diets of Daft *et al.* and Ashburn *et al.*, i.e. *p*-aminobenzoic acid, inositol and vitamin B₁₂. We therefore conclude that the omission of pantothenic acid from the diet has a small effect on haematopoiesis in rats when the diet is fully supplemented by the other known vitamins of the B complex.

SUMMARY

1. One hundred and one black-and-white rats were fed from weaning or from 7 days after weaning on a purified diet with all vitamin supplements except pantothenic acid. They were dosed daily with 25, 50 or 100 μg pantothenic acid either after 2 weeks' deprivation or from the beginning of the test. Another group of seven animals was maintained for 7–11 weeks on the purified diet without dosing and then given a small dose of pantothenic acid for 4 weeks. The response in growth and blood formation was studied.

2. Very little gradation of response in growth was observed with the daily doses of 25, 50 and 100 μg pantothenic acid.
3. Male rats were more sensitive than female rats in their growth response to pantothenic acid.
4. Although a daily dose of 25 μg pantothenic acid was almost adequate for growth, it did not completely prevent the greying of the black parts of the coat.
5. In rats deprived of pantothenic acid the red-cell count increased and the cells were microcytic and hypochromic. There was no anaemia.
6. In pantothenic-acid deficiency the number of white cells did not change.

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Effect of pantothenic-acid deficiency on certain organs of the rat

BY CYNTHIA D. COMBRIDGE AND ALICE M. COPPING

*Departments of Physiology and Nutrition, Queen Elizabeth College
(University of London), Campden Hill Road, London W. 8*

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Since changes had been observed in some organs of the pyridoxine-deficient rat (Combridge, 1956), it was thought that it would be interesting to examine organs from the pantothenic acid-deficient rat and to compare the two sets of results.

EXPERIMENTAL

The forty-seven rats were from series C₁₅, described in the previous paper (Blunt, Cheesman & Copping, 1957), where details of the treatment will be found. After the blood samples had been taken, the rats were killed by opening the thorax. Portions of