ON THE NUTRITIVE STUDIES OF PANTOTHENIC ACID BY ANTIMETABOLITES III. PANTOTHENIC ACID DEFICIENCY PRODUCED BY ω-METHYL PANTOTHENIC ACID IN RATS

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III. PANTOTHENIC ACID DEFICIENCY PRODUCED BY ω-METHYL PANTOTHENIC ACID IN RATS

By

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Drell and Dunn (1) were the first to report on the function of ω-methyl-PaA* as pantothenic acid antimetabolite to many strains of lactic acid bacteria; subsequently they discovered (2) that this substance was also the cause of pantothenic acid deficiency in mice, thus acting as antimetabolite to mammals, too.

In 1954 Bean and Hodges reported that a pantothenic acid deficiency syndrome could be produced in an adult male by feeding him on a pantothenic acid deficient diet added with this antimetabolite(3, 4). Meanwhile the present authors have revealed that this antimetabolite also acts as pantothenic acid antimetabolite on the development of the chick embryo and the germination of many species of higher plants (5, 6).

This paper presents the results of investigations on the histological effect of pantothenic acid-deficient diet containing this antimetabolite mainly on the adrenal and stomach of a rat which has been fed on that diet.

Experiment

(1) Experimental animal

Wister rats (male, weighing 100—140 g) were used for the test. They were bred each in a wire-net cage.

(2) Experimental diet

Food and water were supplied ad libitum. The diet was prepared as a combination of basal food and vitamin tablets with the composition shown in Table 1 to the ratio of 100 g (basal food) to 20 tablets. The control group was

* ω-Methyl PaA : ω-Methyl Pantothenic acid.

put on PaA-Ca 2 mg/100 g diet, the deficient group on PaA-free diet, and the antagonist group on ω-methyl-PaA-Ca 100 mg/100 g diet.

Table 1. Composition of basal diet and vitamin tablet.

(1) Basal diet

Vitamin-free casein (refined by alcohol extraction)	20%
Carbohydrate	60%
McCollum salts	4%
Oil (Hard oil)	10%
Talc	5%

(2) Vitamin tablet (vitamin content per one tablet)

B_1	20 γ
B_2	30 γ
B_6	25 γ
B_{12}	0.05 γ
Choline	5 mg
PABA	250 γ
Folic acid	2.5 γ
Inositol	1.5 mg
Niacin	100 γ
Biotin	0.5 γ
A	150 I.U.
D_2	15 I.U.

Tablet for the control group; PaA-Ca 100γ/1 tablet was added.
Tablet for the deficient groug; No addition
Tablet for the antagonist group; ω-Methyl-PaA-Ca 5mg/1 tablet was added.

(3) *Measurement of acetylation capacity*

On the 21st day of feeding on experimental diets, four rats each from the respective groups were picked for comparison of acetylation capacity. Each of them was intraperitoneally injected with 6 mg of PABA*, the urine excreted within 24 hours of the injection was collected and the total-PABA and free-PABA in it were quantitatively analysed according to Bratton-Marshall's method (7); and assuming that the difference between the measured values represented acetylated PABA, the percentage of acetylated PABA in the total PABA was taken as the indication of acetylation capacity.

(4) *Histological inspection*

On the 33rd day of breeding, the rats were killed and dissected; and each organ was stained for inspection with Hematoxyline-Eosine or Sudan III.

* PABA: *p*-Aminobenzoic acid.

Results

The rats in the deficient group ceased to grow from about the secod week and tended to lose weight slightly, showing, however, no apparent signs of deficiency disease, while those in the antagonist group on ω-methyl-PaA added diet began to lose weight in about a week and to manifest a pantothenic acid deficiency symptom after the second week; their external appearance in the

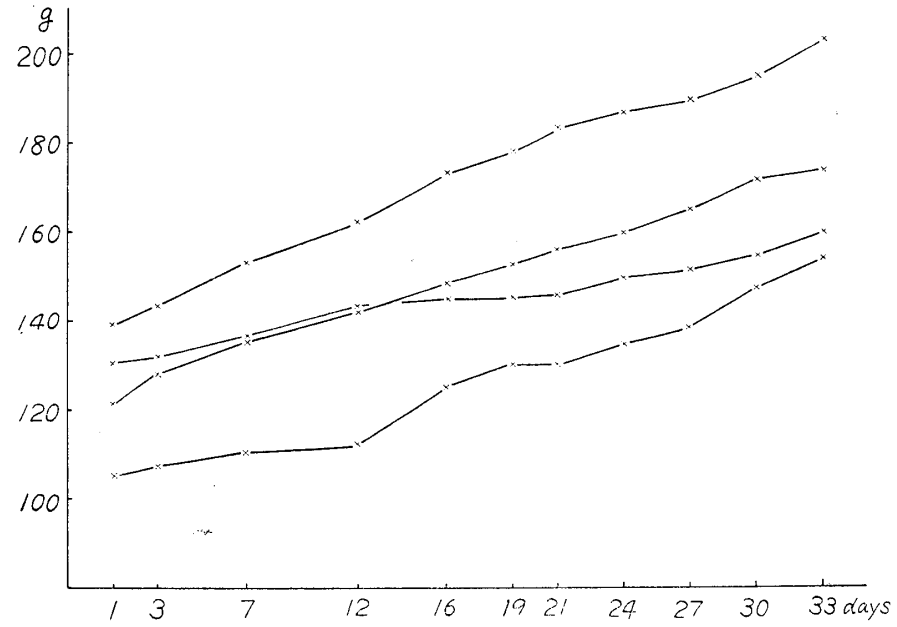

Fig 1. Growth of the Rats. (Control)

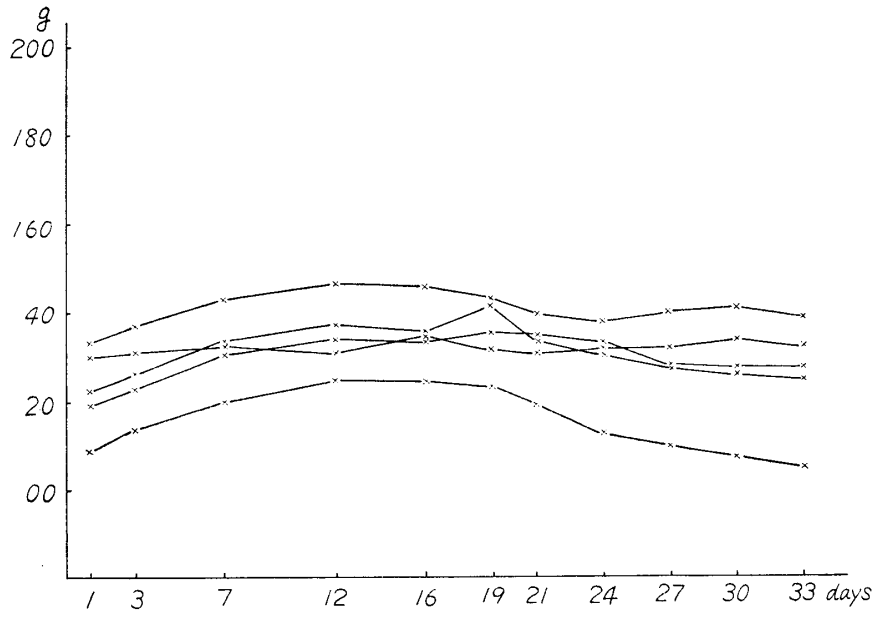

Fig 2. Growth of the Rats. (Deficient)

third week with their hair thinning and turning rusty red was decidedly different from that of the rats in the control group. Some developed diarrhea and in general they became mad; three perished suddenly with convulsion before being killed. Meanwhile, the rats in the control group registered an increase in weight up to the moment of killing. Figs. 1 to 4 plot the weight fluctuation curves of the experimental animals. Table 2 gives the PABA acetylation capacity of the rats in the respective groups on the 21st day of breeding; the capacity of

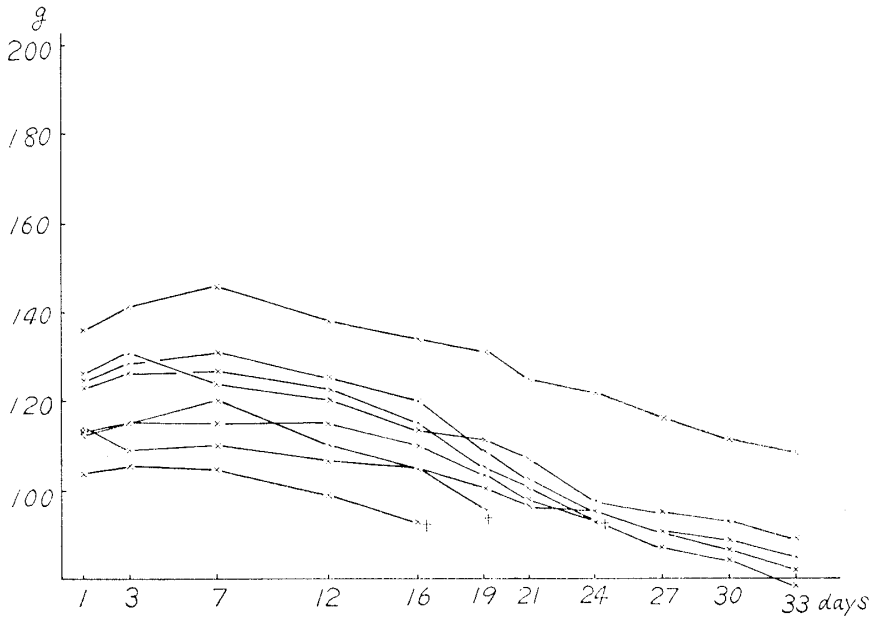

Fig 3. Growth of the Rats. (Antagonist)

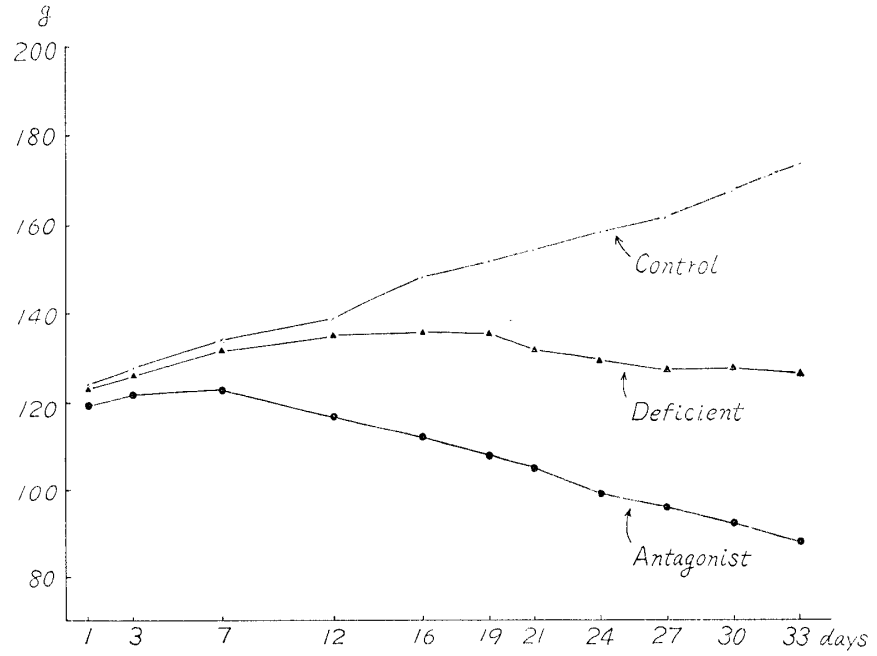

Fig 4. Growth of the Rats.

the deficient group is obviously inferior to that of the control group and that of the antagonist group is more inferior to the latter, testifying to the decline of CoA in their bodies.

Table 2. PABA acetylation capacity.

Group	Weight	Acetylation capacity
Control	156 g	62 %
	146	53
	183	52
	130	50
	Mean value	54.2
Deficient	140	42
	135	45
	134	48
	130	42
	Mean value	44.2
Antagonist	125	44
	107	37
	96	39
	97	42
	Mean value	40.5

Six mg of PABA was injected intraperitoneally; the total PABA and free-PABA in the urine excreted within 24 hours of injecion were measured and the difference between the two was assumed to indicate the acetylated PABA.

On the 33rd day of breeding, all the rats were killed. Upon dissection, the majority of the rats in the antagonist group were found to show signs of hemorrhage in the glandular stomach, therefore they were submitted to more elaborate histological observations. The observations revealed that the stomach wall was about half as thich as that of the rats in the control group; with signs of hemorrhage and atrophy it was histologically in the initial stage of ulcer development. In the rats of the deficient group no marked change was observed, but as compared with the control group, they had their stomach wall thinned.

Except for the glandular stomach the most remarkable change was suffered by the adrenal. Namely, in the great majority of the rats in the antagonist group visible signs of hemorrhage appeared. Histological studies as shown in photo revealed that the rats in the deficient group marked the most conspicuous decrease in the Sudan III-positive substance in the zona reticularis and a substantial decrease in it in the zona fasciculata, too. However, in the zona glomerulosa the Sudan III-positive substance was present in considerable

quantities, which means that lipid grains diminished from zona glomerulosa toward medulla. On the contrary, the rats in the antagonist group had a conspicuous decrease in the lipid grains not only in the zona fasciculata but also in the zona glomerulosa (Plates 1—3). Inspection of the adrenal ortex in the rats which perished suddenly before killing disclosed that each zona was lacking in lipid.

Discussions

Studies on the pantothenic acid deficiency in weanling rats have been reported by many authors.

It is generally accepted that adult rats are hardly subject to such deficiency (8, 9). In the present experiment using relatively mature rats weighing 100 to 120 g, the rats in the deficient group did not exhibit so definite signs of this disease in their appearance. But from that they ceased to grow with their PABA acetylation capacity apparently declining, it is obvious that the physical function in which pantothenic acid takes part has become less active. There is a histological observation which endorses this presumption. Namely, in the rats in the deficient group, the grains of Sudan III-positive substance in the adrenal cortex hardly diminished in the zona reticularis and to a considerable extent, too, in the zona fasciculata, but were found nearly as many in the zona glomerulosa as in the rats in the control group. This was already pointed out by Deane and Mckibbin (10). Meanwhile, the rats in the antagonist group exhibited in two to three weeks external signs which were deemed indicative of pantothenic acid deficiency. Naturally, the PABA acetylation capacity deteriorated in a great measure. Histological observations on them produced interesting discoveries. First, a sharp decrease of lipid in the adrenal cortex; in all the experiments including this one no case was observed where the lipid decrease in the adrenal of rats fed on pantothenic acid deficient diet did extend to the zona glomerulosa. In this experiment the lipid decrease in the adrenal cortex of the rats in the antagonist group extended to the zona glomerulosa; and in the several rats of this group which met sudden death the above mentioned extension of lipid decrease to the zona glomerulosa was invariably observed.

Second, in most of the rats in the antagonist group the glandular stomach developed ulcer-like changes. In the light of the report (11) that a pig suffered ulcer in an experiment on pantothenic acid deficiency in pigs or the report (12) that a rat developed duodenum ulcer due to pantothenic acid deficiency, it would be unreasonable to attribute the ulcer development of glandular stomach in the present experiment to a specific toxic effect of ω-methyl-PaA. Harley *et al.* (13) stated that pantothenic acid deficiency in itself exerts a stress on the adrenal cortex of rats, thereby causing exhaustion and functional deterioration of the adrenal cortex. The ulcer change in the glandular

stomach in the present case may be attributed to a similar cause. From these observations, it may be assumed that ω-methyl-PaA is the cause to severe development of pantothenic acid deficiency in rats. As for the suspicion of a specific toxic effect of ω-methyl-PaA, further investigation will be required to clear up the question.

Summary

An experiment was conducted in which adult male rats were put on a pantothenic acid deficient diet and a diet added with ω-methyl-PaA, which is an antagonist to pantothenic acid for the possible development of pantothenic acid deficiency syndrome. The results are:

(1) The rats on a pantothenic acid deficient diet (deficient group) never developed any clear syndrome of deficiency, but those on ω-methyl-PaA containing diet exhibited apparent syndrome of pantothenic acid deficiency: thinning and turning rusty-red of hair, diarrhea and mad behavior.

(2) Whether the external signs of deficiency appeared, the PABA acetylation capacity deteriorated in both the deficient and the antagonist group.

(3) Most of the rats in the antagonist group suffered ulcer-like development in the glandular stomach, which was histologically confirmed. The rats in the deficient group did not suffer such definite development, but their stomach wall was found to be thinner than that of rats in the control group.

(4) The decrease of Sudan III-positive substance in the adrenal cortex in the deficiency group was the most remarkable in the zona reticularis, followed by the zona fasciculata; but not so great in the zona glomerulosa. By contrast, in the antagonist group, the decrease in this substance not only covered the zona reticularis and the zona fasciculata, but even extended to the zona glomerulosa, showing a sharp decrease.

(5) Some rats in the antagonist group met sudden death, but even in these unfortunate ones no exceptional histological findings were obtained in their glandular stomach and adrenal.

(6) The possibility of ω-methyl-PaA causing pantothenic acid deficiency in adult rats and the process of detecting the syndrome are discussed.

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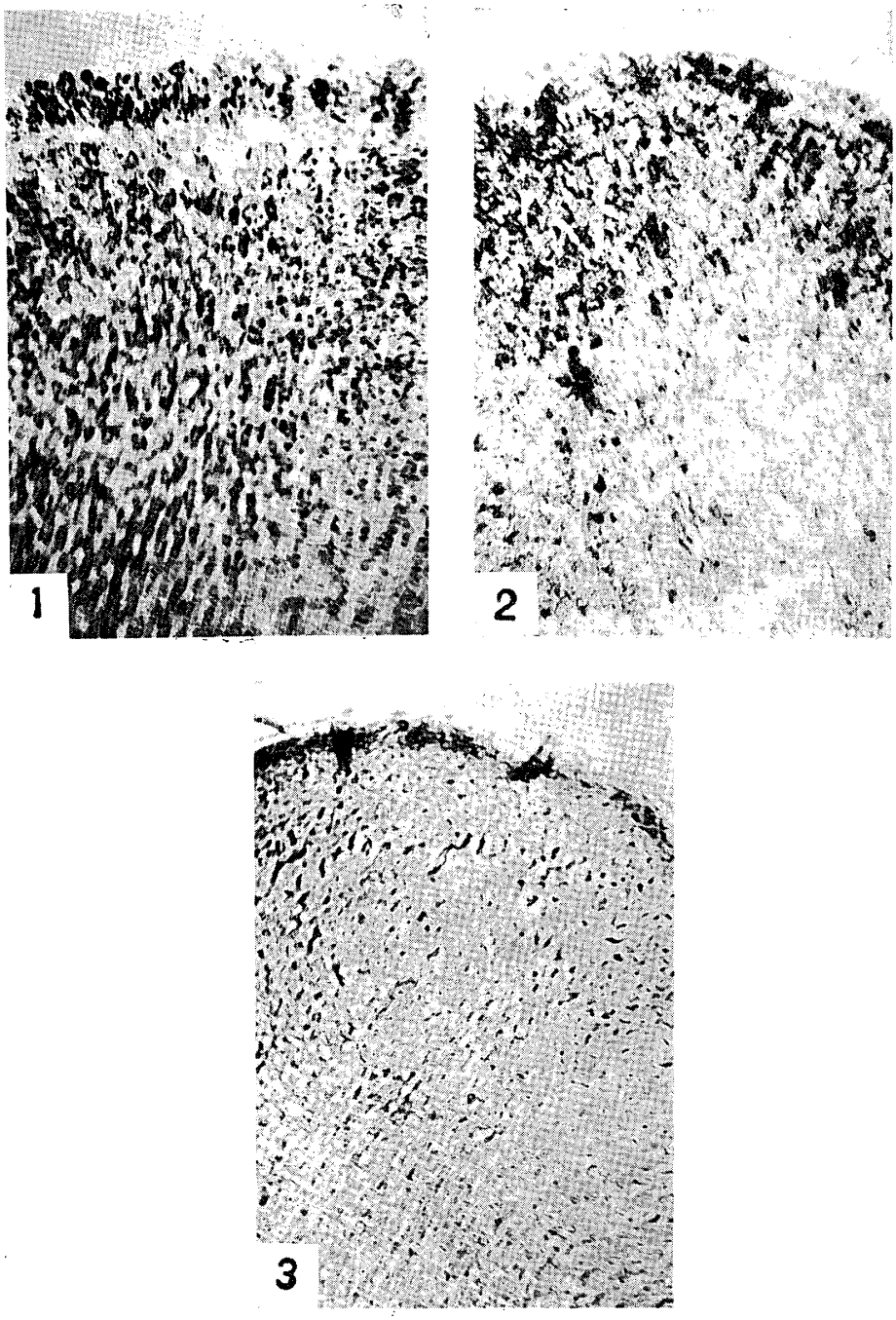

Explanation of plate

Frozen sections of adrenal glands stained with Sudan III.
1. From a rat on the control diet.
2. From a rat on a diet deficient in pantothenic acid.
The decreased lipid content of the inner zona fasciculata and the zona reticularis are seen.
3. From rat on a diet containing the pantothenic acid antagonist.
The lipid disappear clearly in all the zona glomerulosa, the zona fasciculata and the zona reticularis except the capsule.