tions substantiated the gross picture (Fig. 1). This photograph shows the dense leukocytic infiltration by the euglobulin fraction of the extracts. In some instances thrombosed lymphatics and swollen bundles of collagen were observed. In contrast, the pseudoglobulin-albumin fractions induced on microscopic study a relatively mild reaction (Fig. 2). The thermolability and the severely injurious property of the euglobulin fraction of the several organ extracts studied suggest strongly that here again we are dealing with a substance similar to the necrosin recovered from inflammatory exudate. Inflammation is a manifestation of severe cell injury. Crushing tissues mechanically performs the same operation as an irritant. This yields a similar substance from cells that have been severely injured.

Conclusions. The crushing and mashing of cells from dog kidney, liver, and spleen yields by $(NH_4)_2SO_4$ fractionation a necrotizing substance located in the euglobulin fraction of the saline extracts. The pseudoglobulin-albumin fraction of the tissue extracts do not contain this toxic substance. This substance is thermolabile. It does not seem to differ in its biological property of inducing severe cell injury from necrosin as obtained especially from acid inflammatory canine or human exudates(1,2).

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Pantothenic Acid Deficiency and Conjugation Reactions. III. Inhibitory Effect of ω-Methylpantothenic Acid.* (18195)

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The decreased acetylation of aromatic amines by the pantothenic acid deficient rat was reported(1,2) following the discovery that pantothenic acid is a constituent of coenzyme A(3). As part of our studies of factors influencing the acetylating ability of deficient rats, we report here on some of the effects of N(α,γ-dihydroxy-β,β-dimethylvaleryl)-β-alanine, termed ω-methylpantothenic acid† by Drell and Dunn(4). Omega methylpantothenic acid was first tested by Nease (5) and reported to show no growth-promoting activity for *Lactobacillus casei.* Drell and Dunn(4,6) noted that it inhibited the growth of a number of lactic acid bacteria. More recently, Drell and Dunn(7) have demonstrated reversible inhibition of growth with mice using this analog. Inhibition of growth of rats by the analog was prevented by simultaneous administration of pantothenic acid (8). Before the latter reports were available we decided to test this compound for its possible effects on mammals with particular reference to its influence on the acetylation of sulfanilamide by pantothenic acid deficient

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1. Riggs, T. R., and Hegsted, D. M., *J. Biol. Chem.*, 1948, v172, 539.

2. Shils, M. E., Seligman, H. M., and Goldwater, L. J., *J. Nutrition*, 1949, v37, 227.

3. Lipmann, F., Kaplan, N. O., Novelli, G. D., Tuttle, L. C., and Guirard, B. M., *J. Biol. Chem.*, 1947, v167, 869.

† We are indebted to Dr. Max S. Dunn, Dept. of Chemistry, University of California at Los Angeles for the sodium salt of ω-methylpantothenic acid. According to Dr. Dunn (personal communication) sample 1 contained 3% of impurities and sample 2 contained 7%, presumably the lactone portion.

4. Drell, W., and Dunn, M. S., *J. Am. Chem. Soc.*, 1948, v70, 2057.

5. Nease, A. H., *Dissertation*, U. of Texas, 1943, quoted by Drell and Dunn(6).

6. Drell, W., and Dunn, M. S., *J. Am. Chem. Soc.*, 1946, v68, 1868.

7. Drell, W., and Dunn, M. S., Absts. 115th meeting ACS, San Francisco 1949, p. 13c

8. Dunn, M. S., personal communication, July 1950.

rats before and after a given amount of calcium pantothenate. Such a procedure appears to offer a specific biochemical test for pantothenic acid analog activity and to allow results to be obtained in a short time and with much smaller amounts of the analog than would be required for growth inhibition studies.

Experimental. The acetylation test was similar to that previously reported(2). Sulfanilamide (0.8% solution) was injected intraperitoneally at 3 successive hourly intervals in amounts of 24 mg, 16 mg, and 16 mg, respectively. A 22 hour urine collection was begun immediately after the first injection. Free and total sulfanilamide was determined by the method of Bratton and Marshall(9) and the results expressed as the percentage of the total urinary sulfanilamide acetylated.

Preliminary trials with various amounts of the first sample† of sodium ω-methylpantothenate, given by intraperitoneal injection, indicated that the compound was capable of inhibiting the usual acetylation and growth response of pantothenic acid deficient rats‡ given a single intraperitoneal injection of 2 mg of calcium pantothenate. With single 15, 10, 10, and 15 mg doses of the analog given on 4 successive days, 3 of 6 animals failed to respond to the calcium pantothenate given 3 hours before the start of the acetylation test. In another trial with 4 deficient rats 50 mg of the analog were given 1½ hours before injecting 2 mg of calcium pantothenate; another 50 mg of the analog were given the following day and 2½ hours later the acetylation test was started. No response to the vitamin was noted either biochemically or in growth during the following 4 days. With these preliminary data offering some basis for dosage, a more detailed experiment was performed employing the second sample† of sodium ω-methylpantothenate. The rats were fed diet 10-P‡ until they were at a state of deficiency where weight gain had ceased and acetylation of sulfanilamide was reduced to 50% or less of normal values. These animals, which were approximately equal in number between the sexes, were divided into 3 groups. Group 1 received the analog for 3 days (a total of 140 mg) while group 2 received it for 6 days (a total of 230 mg); the acetylation responses with and without calcium pantothenate were noted at intervals. Group 3 served as a control and did not receive the analog. Details of treatment are given in Table I; diets which were started on a given day were continued unless otherwise noted. Results of the acetylation tests are summarized in Table II. For comparative purposes there are included the acetylation data of tests given 6 days (Day-6) and one day (Day-1) before analog administration.

It is evident that, while the analog was being administered, the usual response in acetylation following calcium pantothenate administration to deficient rats was prevented (compare Groups 1 and 3 on day 2 and Group 2 on day 6). It will be noted that there was a small increase in the per cent acetylation by Group 1 on day 2 over the acetylation in the preceding test; this did not occur with Group 2, perhaps because of the more prolonged analog administration. When acetylation was tested again (day 8 or 9) after an interval without the analog the per cent acetylation had decreased (Groups 1 and 2). Finally, the animals were tested for their ability to respond to the vitamin after a period without any analog during which calcium pantothenate was ingested and a return to control values was noted (day 15 or 20).

Discussion. Because of the failure of other pantothenic acid analogs to exhibit anti-vitamin activity in mammals, ω-methylpantothenic acid assumes special interest. Its inhibiting action on acetylation *in vivo* may be due to (a) anti-vitamin action, (b) a

9. Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, v128, 537.

‡ The pantothenic acid deficient diet (Diet 10-P) had the following percentage composition: casein (Labco vitamin free) 18, cerelose 66, corn oil 10, salts (USP No. 2) 4, plus vitamins added per 100 gm of basal diet: thiamine.HCl 1 mg, riboflavin 1 mg, pyridoxine.HCl 1 mg, niacin 2 mg, folic acid 0.20 mg, biotin 0.002 mg, choline 100 mg, and percomorph oil 0.12 ml. Diet 10 had the composition of diet 10-P with calcium pantothenate added at a level of 5 mg %.

TABLE I. Schedule of Treatment of 3 Groups of Rats in Testing Effect of Sodium ω-Methylpantothenate

Day	Group 1	Group 2	Group 3
0	25 mg analog*; diet 10-P + ½% analog†	As in Gr. 1	H_2O; diet 10-P†
2	9 a.m.-25 mg analog	As in Gr. 1	9 a.m.-H_2O
	11 a.m.-2 mg Ca pantothenate	11 a.m.-H_2O	As in Gr. 1
	2 p.m.-acetylation test	As in Gr. 1	As in Gr. 1
3	Diet 10-P	Diet 10-P + ½% analog*	As in Gr. 1
6	—	As in Gr. 1 on Day 2	—
8 or 9	Acetylation test	As in Gr. 1 "	As in Gr. 1
10	2 mg Ca pantothenate; diet 10	As in Gr. 1 "	As in Gr. 1
15 or 20	Acetylation test	As in Gr. 1 "	As in Gr. 1

* The 25 mg of Na ω-methylpantothenate, 2 mg of Ca pantothenate and water were inj. intraper. in volumes of 1 ml.

† Restricted to 6 g daily.

TABLE II. Influence of Sodium ω-Methylpantothenate on Acetylation Response to Calcium Pantothenate by Pantothenic Acid Deficient Rats.*

Group No.	No. of rats	Day†					
		−6	−1	2	6	8 or 9	15 or 20
1	8	12.6 ± 1.04‡	13.2 ± 1.02‡	18.0 ± 1.18§	—	12.3 ± 1.24	33.4 ± 1.00
2	6	16.5 ± 2.41‡	17.9 ± 2.72‡	15.9 ± 2.04‖	16.9 ± 1.55§	10.6 ± 1.83	34.9 ± 1.82
3	7	17.4 ± 1.40‡	17.4 ± 0.81‡	31.0 ± 1.13¶	—	29.6 ± 0.97**	35.1 ± 1.20

* Mean ± stand. dev. of the mean of the % acetylation of sulfanilamide excreted in acetylation test.

† See Table I for schedule of treatment.

‡ No treatment.

§ Analog and vitamin given.

‖ Analog given.

¶ Vitamin given.

** Only 4 of the 7 rats of group 3 were tested at this time.

generalized toxic effect, or (c) a specific toxic effect on some phase of acetylation not directly involving pantothenic acid. While possibilities (b) and (c) cannot be ruled out at this time, the analog mechanism is probably the operative one. If this is correct, then when given prior to the vitamin, ω-methylpantothenic acid is either interfering with the adequate formation of co-enzyme A or its proper association with its apoenzyme. The mechanism of action of the analog will be clarified by further study of the concentration relationships between the analog and the vitamin in affecting acetylation and growth and the concentration of coenzyme A in the tissues.

Summary. An analog of pantothenic acid, the sodium salt of N(α,γ-dihydroxy-β,β-dimethylvaleryl) β-alanine (termed ω-methylpantothenic acid) has been found to prevent pantothenic acid deficient rats from responding normally to calcium pantothenate as measured by an acetylation test using sulfanilamide. When 140 mg of the analog were administered over a period of 3 days only a small response was noted; 230 mg over a six-day period prevented any response. After the analog was withdrawn and pantothenic acid administered, the animals exhibited a marked increase in acetylation.

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