ment of immature male rats under cold room conditions. The protective effect of these supplements, however, was no greater than that provided by the synthetic B vitamin supplements. Since previous findings(7) indicate that supplements of the known B vitamins (excepting vit. B_{12}) were without significant effect on the growth of immature rats fed a ration similar to diet A it would appear that the vit. B_{12} content of diet B was responsible at least in part for its protective effect under conditions of low environmental temperature.†

Summary. A marked retardation in body and gonadal weight was observed in immature male rats fed a purified ration under conditions of low environmental temperature. Supplements of desiccated whole liver, water-insoluble liver residue or water-soluble liver extract resulted in a marked increase in both body and gonadal weight. A supplement of the known B vitamins was similarly effective. It is suggested that the protective effects of the various supplements were due, at least in part, to their vitamin B_{12} content.

† According to data supplied by the manufacturers Whole Dried Liver Powder contained approximately 1.1 mcg vit. B_{12}-like activity per g, Extracted Liver Residue from 0.2 to 0.4 mcg/g and Liver Concentrate Powder 1-20 from 3.0 to 5.0 mcg/g. Since the whole liver and liver residue were fed at a 10% level in the diet and the liver concentrate at a 2.5% level, the vit. B_{12} content of diets C,D and E were approximately 110 mcg per kg of diet, 20 to 40 mcg per kg of diet and 75 to 125 mcg per kg of diet respectively.

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Toxicity and Effect of ω-Methylpantothenic Acid on Blood-Induced Infections of Plasmodium Lophurae in the Chick.* (18154)

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The inhibitory activity of certain analogs of pantothenic acid toward a variety of organisms has attracted attention during recent years. Several analogs, notably derivatives of pantoyltauramide and phenylpantothenone, have been found active for some strains of plasmodia(1,2). The relatively high bacteriostatic effect of ω-methylpantothenic acid [N-(α, γ-dihydroxy-β,β-dimethylvaleryl)-β-alanine](3) prompted the present investigation on the antiplasmodial potentialities of this compound. The toxicity of this compound for the test animal, the chick, was extended to include its effect on rats, mice and on *Tetrahymena geleii* H.

Toxicity studies. Chicks. Preliminary experiments on a total of 20 chicks, 1-2 weeks old and weighing 53-85 g, showed no toxicity of single doses of sodium ω-methylpantothenate given orally in concentrations as high as 8.4 g/kg body weight, intravenously in concentrations up to 0.4 g/kg and intraperitoneally in concentrations up to 1.0 g/kg. The analog (80% pure) was administered as a neutral 30% solution. Ten birds injected intraperitoneally with higher single doses (3.2-4.5 g/kg) exhibited torpor, loss of appe-

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1. Wiselogle, F. Y., Editor, A Survey of Antimalarial Drugs, 1941-1945. J. W. Edwards Co., Ann Arbor, Mich.

2. Brackett, S., Waletzky, E., and Baker, M., *J. Parasit.*, 1946, v32, 453.

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

TABLE I. Response of *Tetrahymena geleii* H to ω-Methylpantothenic Acid.

Sodium ω-methyl-pantothenate, γ/tube	Calcium pantothenate,† γ/tube	Incubation time in days: Slanted tubes 6 O.D.*	Slanted tubes 9 O.D.	Slanted tubes 14 O.D.	Upright tubes 9 O.D.	Upright tubes 14 O.D.	Upright tubes 17 O.D.	Upright tubes 21 O.D.
0	0	.80	1.11	1.39	.45	.93	1.14	1.20
20	0	.77	1.09	1.33	.54	.99	1.13	1.18
200	0	.87	1.24	1.70	.42	.90	1.12	1.12
2000	0	.48	.97	1.55	.40	.72	1.05	1.13
20000	0	.28	.42	0.76	.21	.30	0.44	0.67
0	5				.42	0.90	1.15	1.16
20	5				.66	1.04	1.20	1.17
200	5				.58	0.97	1.18	1.16
2000	5				.54	0.97	1.23	1.26
20000	5				.30	0.53	0.80	1.01

* Optical density × 10, measured with a Lumetron photoelectric colorimeter, model 400A, using a red (650 mμ) filter and corrected for the inoculated tubes at zero time. Values given are averages of duplicate tube readings.

† Added aseptically (0.1 ml) on the 6th day.

tite and inability to control equilibrium but all of the chicks recovered after 8-10 hours. Daily intraperitoneal injections of 0.45-0.50 g kg for 5 successive days produced no demonstrable toxicity but when a larger dose (3.2-4.4 g/kg) was given to 5 birds for 6 successive days, 3 of the chicks died within 7 days and the remaining 2 succumbed on the 11th and 15th days. The average increase in weight of the treated birds on the 6th day was 16% compared to 35% for the controls. Reticulocyte counts of the treated chicks were not increased appreciably over the normal percentage of reticulocytes found in the peripheral blood of untreated 1-2-week-old chicks.

Rats. Eight rats of the Long-Evans strain 5-6 weeks old and weighing 120-150 g were given a single dose of sodium ω-methylpantothenate orally via a catheter at a level of 10 g/kg body weight. The pure analog (N,calcd. 5.49; found, 5.46) was dissolved in an equal weight of water and the solution (pH, 10.1) was administered without being neutralized. Depilation was observed at sites where the solution accidently came in contact with the fur. The toxic manifestations observed were rapidly-developing torpor, loss in appetite for many hours and diarrhea which was mild in 3 cases and severe in 5 others. Some of the experimental animals failed to gain weight in 3-5 days and others lost weight. A single intraperitoneal injection of the pure analog was administered unneutralized as a 10% solution (pH, 10.3) at a level of 1.0 g/kg to 8 rats 7-9 weeks old and weighing 180-240 g. This resulted in reduced activity but no other symptoms. On the other hand, following supplementation of a pantothenic acid-deficient diet with the analog at a level of 0.5%, marked loss in weight and death of weanling 28-day rats occurred within 2-3 weeks. This effect was prevented by inclusion in this diet of calcium pantothenate (.01%).

Mice. Eight young albino mice of the Bagg strain weighing 20-22 g recovered completely after a single oral dose of sodium ω-methylpantothenate at a level of 10 g/kg. The analog was prepared and administered in the same manner as for rats. The torpor was somewhat less marked than that observed with rats. There were no marked toxic symptoms when the analog was given once intraperitoneally to 8 Bagg mice at a level of 0.9 g/kg or to 10 animals at 1.0 g/kg. A deficiency syndrome resulting from the daily ingestion of the analog was prevented or reversed by pantothenic acid(4).

Protozoa. The inhibition of growth of *Tetrahymena geleii* H by ω-methylpantothenic acid was reversed by pantothenic acid as shown in Table I. The procedure and basal

4. Drell, W., and Dunn, M. S., Abstr. of Papers, 115th Meeting, Am. Chem. Soc., 1949, p. 13C.

TABLE II. Effect of Intraperitoneal Administration of ω-Methylpantothenic Acid on *P. lophurae* in Chicks.

No.	Parasitemia in days (parasitized cells/10,000 rbc)							
	2	3	4	5	6	7	8	9
				Experimental				
A1	570	1210	2280	3520	2560	2250	130	—50
2	620	1400	2480	3820	2730	1320	140	—50
3	740	1620	3060	4540	3320	1610	210	—50
4	480	1050	2020	2980	2350	920	110	—50
5	540	1180	2260	3340	2430	1220	140	—50
Avg	590	1292	2420	3640	2675	1244	146	—50
				Control				
CA1	650	1520	2700	4100	2920	1410	160	—50
2	580	1310	2420	3580	2710	1230	130	—50
3	720	1550	2980	4420	3380	1520	190	—50
4	630	1450	2620	3780	2900	1300	150	—50
5	450	1170	1900	2800	2100	1150	90	—50
6	710	1590	2940	4360	3120	1450	200	—50
Avg	638	1431	2593	3640	2855	1343	153	—50

medium (supplemented with the analog as indicated) was that of Rockland and Dunn (5).§

Effect of ω-methylpantothenic acid on P. lophurae in chicks. Intraperitoneal administration. The second day after inoculation with *P. lophurae* a 2 g/kg dose of the analog was administered twice daily for 4 successive days to 5 week-old chicks maintained on commercial starting chick mash. The strain of *Plasmodium lophurae* used was furnished by Dr. R. H. Rigdon then of the University of Arkansas School of Medicine. It was received in ducklings and maintained by transfer at 6-day intervals to week-old chicks. 0.2 ml of heparinized blood from chicks with at least 40% of red cells parasitized was used for inoculation. Growth and activity were decreased but there was no significant depression of parasitemia compared to 6 control chicks infected simultaneously with a similar inoculum (Table II). Infections, on the other hand, were markedly depressed by oral (400 mg/kg/day) or intraperitoneal (80 mg/kg/day) administration of 0.1% quinine hydrochloride.

Oral administration. The analog (10 g/kg) was administered orally to 5-week-old chicks on the day preceding inoculation with *P. lophurae* and treatment with the analog (5 g/kg/day) was continued for 5 successive days. No depression of parasitemia was observed in the treated compared to the (5) control chicks.

Effect of pantothenic acid deficiency on P. lophurae in chicks. The inactivity of ω-methylpantothenic acid toward *P. lophurae* suggested further study regarding the metabolic need of the parasite for pantothenic acid. Trager(6) demonstrated that pantothenic acid deficiencies do not alter the course of infections with *P. lophurae* in chicks and ducks, but these birds also had a concomitant biotin deficiency. Brackett, Waletzky and Baker(2) on the other hand, reported that trophozoite-induced infections of *P. gallinaceum* in chicks were strikingly less severe in pantothenate-deficient birds than in supplemented control chicks, suggesting that the blood phases of gallinaceum malaria require pantothenic acid for growth and multiplication. Inasmuch as the analog in question has been found to be active only against organisms which require pantothenic acid preformed in their media(7), it was thought expedient to repeat a portion of Trager's work by subjecting

5. Rockland, L. B., and Dunn, M. S., *J. Biol. Chem.*, 1949, v179, 511.

§ The medium was modified by omitting pantothenic acid and liver extract and by increasing the level of amino acids about 50%. The authors are indebted to Dr. L. B. Rockland and Mr. Jose Lieberman for suggestions and assistance.

6. Trager, W., *J. Exp. Med.*, 1943, v77, 557.

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

TABLE III. Blood-induced Infections of *P. lophurae* in Pantothenic Acid-Deficient Chicks.

Diet*	Age in days	Wt in g	% parasitized red cells in indicated days							
			2	3	4	5	6	7	8	9
A	15	63(7)†	3.5	6.0	12.4	28.0	21.0	12.0	2.0	−1.0
			(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)
B	15	63(5)	3.0	6.8	12.0	31.0	24.0	10.0	1.0	−1.0
			(5)	(4)	(3)	(3)	(3)	(2)	(1)	(1)
C	15	102(5)	5.0	7.9	16.5	36.1	38.0	25.0	10.0	5.0
			(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

* Symbols defined in text.
† Figures in parentheses indicate number of birds on which averages are based.

pantothenic acid-deficient chicks to infection with *P. lophurae*. The 3 diets employed as indicated in Table III were (a) Diet A, essentially the vitamin-free diet of Brackett *et al.*(2), (b) Diet B, Diet A containing 1% of ω-methylpantothenic acid and (c) Diet C, Diet A containing 0.6 mg% of calcium D-pantothenate. Although the concentration of pantothenic acid was slightly below the optimal according to Hegsted and Riggs(8), significant deficiency effects probably would not occur during the short time of the experiment. Each group of one-day-old chicks was fed one of the three diets *ad libitum*. Eight of the 12 birds on the deficient diets exhibited by the 15th day eyelid symptoms symptomatic of pantothenic acid deficiency. The weight of the chicks on the experimental diets averaged only about half of that of the animals on the control (C) diet. On the 15th day, the control and deficient chicks were inoculated intravenously with the parasite in doses commensurate with the weights of the birds. The basic dosage was 0.2 ml of blood with 40% R.B.C. infected, for a chick of 50-60 g. Four of the 5 chicks on the analog-supplemented deficient diet died within 22 days after initiation of the regimen.

As shown in Table III, the number of parasitized red cells was only slightly decreased in the birds on the deficient Diet A during the early stages of the parasitemia. By the sixth day, however, the chicks on both diets A and B had a lower percentage of parasitized cells than did those on diet C.

Discussion. It is noteworthy that sodium ω-methylpantothenate has extremely low acute toxicity‖ (Table IV). The rapid recovery of the animals after single large doses of the analog indicates a high rate of excretion. Levels high enough to interfere with the utilization of the tissue pantothenic acid can be maintained only by continued ingestion of the compound. Simultaneous supplementation with pantothenic acid prevents all symptoms associated with the analog, and addition of the vitamin during the course of the drug administration reverses its effects. It appears, therefore, that any inherent toxicity of the analog is of minor significance and that the observed effects are explained by interference with the utilization of the vitamin.

TABLE IV. Acute Toxicity* of Calcium Pantothenate and Sodium ω-Methylpantothenate.

Animal	Na ω-methyl-pantothenate		Cal. pantothenate†	
	Oral	Intra-peritoneal	Oral	Intra-peritoneal
Mouse	10	.92	>10	>1
Rat	>10	.82	>10	>1
Chick			>8.4	>4.5

* LD_{50} in g/kg body wt.
† Data of Unna and Greslin(9).

The response of *Tetrahymena geleii* H to ω-methylpantothenic acid and its reversal by the vitamin probably signifies a requirement for pantothenic acid since to date few exceptions have been noted in *in vitro* experiments to the finding that reversible susceptibility to the analog is correlated with a vitamin re-

8. Hegsted, D. M., and Riggs, T. R., *J. Nutr.*, 1949, v37, 361.

‖ ω-methylpantothenic acid at high levels is nontoxic to lactic acid bacteria in media containing adequate pantothenic acid(3).

9. Unna, K., Greslin, J. G., *J. Pharm. Exp. Therap.*, 1941, v73, 85.

quirement(10). The lack of response of the organism to added pantothenic acid and the relatively high level of analog required to cause inhibition indicate that the pantothenic acid or an active derivative in the natural supplement (cerophyl extract) of the medium was adequate for growth. These experiments were carried out prior to and agree with the report of Kidder and Dewey(11) on strain W of *T. geleii.*

Numerous possible anti-plasmodial agents have been tested following the report of Trager(12) that the *in vitro* survival of *Plasmodium lophurae* was prolonged by calcium pantothenate. Phenylpantothenone has been found to be the most effective analog of the vitamin in protecting chicks from such infections(1). Although it inhibited the growth of all microbial species tested, its effect generally was reversed by pantothenic acid only in organisms requiring the preformed vitamin (13).

According to Novelli and Lipmann(14, 15) both phenylpantothenone and ω-methylpantothenic acid interfere with the synthesis of coenzyme A. Both compounds possess similar inhibitory activities toward lactic acid bacteria(3,13). On the basis of these results, therefore, the protective action of phenylpantothenone against *P. lophurae* in the chick does not appear to be due to interference with pantothenic acid utilization itself. It is still possible that this compound may interfere with the formation of coenzyme A but at a level not subject to reversal by the vitamin and not susceptible to interference by ω-methylpantothenic acid. It has been observed in studies by Brackett *et al.*(2) that the percentage increase of immature erythrocytes produced in the chick by analogs of pantoyltauramide was approximately proportional to the activity of these compounds as anti-plasmodial agents for *P. gallinaceum.* These substances were inactive toward *P. lophurae* in the duck(2). It is of interest that ω-methylpantothenic acid, which is also inactive against *P. lophurae,* failed to produce an increased reticulocyte count in the chick. Cooperative studies by the National Institute of Health have shown that the analog is inactive against *P. cathemerium* in canaries (500-600 mg/kg fed twice daily for 4 days) and against *P. gallinaceum* in week-old chicks (in maximum tolerated doses(1) of 150 mg/kg fed twice daily for 4 days).

The decrease in parasitemia in deficient birds indicated in Table III is possibly due to the lack of pantothenic acid in these diets but this cannot be considered conclusive because of the few animals surviving and the difficulty in making accurate counts in the severely deficient and anemic birds. Becker *et al.*(16) showed that *P. lophurae* was able to mobilize pantothenic acid and/or biotin at the expense of the host. Hence even though blood levels of the vitamin decrease to 25% of normal in deficient chicks(17), this concentration may still be sufficient for initial growth, in contrast to *P. gallinaceum,* whose growth is markedly inhibited in deficient chicks. Trager(6) using *P. lophurae* in chicks found no significant difference between control and pantothenic acid-deficient birds. The heavier infection in birds with calcium pantothenate added to the diet was attributed to a partial biotin deficiency.

Summary. ω-Methylpantothenic acid has been shown to exhibit low acute toxicity toward rats, mice and chicks. Growth inhibition of *Tetrahymena geleii* H at high levels of this analog was reversed by pantothenic acid. *Plasmodium lophurae* infections in chicks on the other hand were unaffected by ω-methylpantothenic acid administered intraperitoneally at a level of 2 g/kg twice daily for 4 days or orally at 5 g/kg daily for 5 days. In chicks on a pantothenic acid-defi-

10. Drell, W., Dissertation, Univ. of Calif., Los Angeles, 1949.

11. Kidder, G. W., and Dewey, V. C., *Arch. Biochem.,* 1949, v21, 66.

12. Trager, W., *J. Exp. Med.,* 1943, v77, 411.

13. Woolley, D. W., and Collyer, M. L., *J. Biol. Chem.,* 1945, v159, 263.

14. Novelli, G. D., and Lipmann, F., *Fed. Proc.,* 1948, v7, 177.

15. Novelli, G. D., personal communication.

16. Becker, E. R., Brodine, C. E., and Marousek, A. A., *J. Infect. Dis.,* 1949, v85, 230.

17. Snell, E. E., Pennington, D., and Williams, R. J., *J. Biol Chem.,* 1940, v133, 559.

cient diet, the simultaneous administration of ω-methylpantothenic acid did not alter the course of the infection with *P. lophurae*.

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Effect of Feeding Aureomycin to Fattening Lambs. (18155)

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The recent work of Stokstad *et al.*(1) with chicks provided the first indication that a growth promoting factor in addition to vit. B_{12} was produced by *Streptomyces aureofaciens*. More recently Stokstad *et al.*(2), in further work with chicks, found that aureomycin produced a growth response when added to diets containing vit. B_{12} and B_{12b}. Similar findings have been reported by Cunha *et al.* (3) and Jukes *et al.*(4) with swine. On the other hand, Colby *et al.*(5), in work with young lambs, found that an animal protein factor concentrate containing aureomycin was harmful. In the trial reported herein, it was decided to test the effects of adding vitamins to the rations of lambs fed aureomycin* in an attempt to overcome these harmful effects. The effect on vitamin levels was indicated in the earlier work with lambs since the blood levels of vitamin B_{12} were found to be lower in those lambs fed the APF concentrate containing aureomycin.

Experimental. Eighteen lambs of mixed breeding weighing an average of about 58 lb were used in this study. They were divided into 3 lots of 6 on the basis of weight and sex. The lambs in Lot I received the basal ration consisting of 90% milo, 10% cottonseed meal plus alfalfa hay, free choice. The lambs in Lot II received the basal ration plus sufficient crude aureomycin to furnish 100 mg of pure aureomycin, daily by capsule. The lambs in Lot III were fed the basal ration plus 100 mg of aureomycin daily by capsule and in addition received an allowance of B-vitamins fed orally by capsule every second day. The B-vitamins fed were as follows (per lamb daily, based on the quantities reported by Lindley *et al.*)(6); thiamine 6.8 mg, riboflavin 1.6 mg, niacin 15.6 mg, pantothenic acid 6.5 mg, pyridoxine 2.6 mg, choline 130 mg, biotin 34 μg, folic acid 169 μg, para-aminobenzoic acid 16.9 mg, and inositol 167 mg. In addition a mixture of vit. B_{12} and B_{12b} was fed at the rate of 20 μg per lamb daily. Feed and weight records were kept. At the close of the trial, 2 lambs from each of the 3 lots were slaughtered, their stomach contents sampled and total bacteria counts made. Bacterial counts were made by the tryptose agar plate method. The cultures were incubated at 37½°C and the total count made at the 48th hour period of incubation.

Results and discussion. The gains and feed efficiency of the 3 lots of lambs used in this trial are shown in Table I. It may be noted that the lambs in Lot I made very satisfactory gains (0.52 lb per head per day)

1. Stokstad, E. L. R., Jukes, T. H., Pierce, J., Page, A. C., Jr., and Franklin, A. L., *J. Biol. Chem.*, 1949, v180, 647.

2. Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 523.

3. Cunha, T. J., Burnside, J. E., Buschman, D. M., Glasscock, R. S., Pearson, A. M., and Shealy, A. L., *Arch. Biochem.*, 1949, v23, 324.

4. Jukes, T. H., Stokstad, E. L. R., Taylor, R. R., Cunha, T. J., Edwards, H. M., and Meadow, G. B., *Arch. Biochem.*, 1950, v26, 324.

5. Colby, R. W., Rau, F. A., and Couch, J. R., *Am. J. Physiol.*, in press.

* The crude aureomycin and vitamins B_{12} and B_{12b} used in this study were obtained through the courtesy of Dr. T. H. Jukes and Lederle Laboratories, Pearl River, N. Y.

6. Lindley, C. E., Brugman, H. H., Cunha, T. J., and Warwick, E. J., *J. An. Science*, 1949, v8, 590.