Chlorotetracycline and Growth Promoting Effect of Beta Carotene and Vitamin A in Rats.* (26123)

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Some of the antibiotics have been found effective in producing additional growth in experimental animals, particularly those experimental subjects maintained under unsanitary conditions and subsisting on diets deficient in some of the water-soluble vitamins. This increased growth rate has been attributed to: (a) partial elimination of the microorganisms from the digestive tract, (b) increased vitamin synthesis in the digestive tract, (c) improved absorption of vitamins and other nutrients, and (d) other factors.

More recent reports indicate that some of the antibiotics when incorporated in the ration also stimulate additional growth in Vit. A-deficient animals and increase liver storage of the vitamin. Almquist *et al.*(1), Burgess *et al.*(2,3) and Coates *et al.*(4) have reported that penicillin and/or chlorotetracycline feeding improved utilization of it. A and/or carotene by the growing chick as indicated by blood level and liver storage of the vitamin. Barber *et al.*(5) reported increased hepatic Vit. A following chlorotetracycline feeding but not following the feeding of penicillin. High(6) reported that chlorotetracycline feeding increased liver and kidney storage of Vit. A in rats when carotene was fed but not when Vit. A was fed. Murray *et al.*(7,8,9) reported that addition of chlorotetracycline to Vit. A-deficient diets decreased the rate of depleting Vit. A reserve in rats, prolonged the survival period and increased the growth rate when restricted intakes of the vitamin were fed. The antibiotic did not affect significantly liver storage of the vitamin over a 4-week period or growth rate when large doses of the vitamin were offered. The increased growth rate was observed when the vitamin was administered either orally or subcutaneously.

On the other hand, Swick *et al.*(10) reported that penicillin feeding did not increase growth rate of rats receiving limited intakes of Vit. A, whereas Hartsook *et al.*(11) reported that dietary supplementation with tetracycline not only failed to exert a sparing effect on Vit. A but actually increased the deficiency syndrome.

Hence, the preponderance of evidence seem to indicate that some of the antibiotics do influence carotene and Vit. A metabolism when taken into the animal body under certain conditions. It appears that further knowledge of the physiological effect of the antibiotics in this particular connection will be helpful in explaining their mode of action in nutrient economy.

Methods. In the initial experiment weanling rats were depleted of their body-stores of Vit. A by maintaining them on the U.S.P. Vit. A-deficient diet.† All rats were caged in individual all-metal cages having raised screen floors. When depleted the rats were arranged into comparable experimental groups and fed the basal diet after incorporating into it varying amounts of chlorotetracycline (Aureomycin).‡ In addition, each rat received by mouth 1 μg beta carotene (in cottonseed oil) daily as the Vit. A supplement. Distilled water was available at all times. The rats were continued under observation for 28 additional days unless death intervened. Body weight and food intake records were recorded and external appearance and actions of the test animals noted.

When it became evident that cholorotetracycline incorporated into a Vit. A-deficient diet exerted a definite growth-promoting effect on Vit. A-deficient rats receiving a sub optimum intake of beta carotene a second series of studies was carried out in which

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† *U. S. Pharmacopeia* XIV, 788.

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TABLE I. Effect of Different Levels of Chlorotetracycline on Growth Rate of Vitamin A-Deficient Rats While Receiving 1.0 μg of Beta Carotene Daily.

Animal group No.	Added chlorotetracycline, g/kg diet	Rats/group ♂	Rats/group ♀	Mean wt of diet consumed,* g	Mean wt gain,* g	Diet consumed/g of gain, g
1	None	31		266	42.6 ± 3.13†	6.24
2	.250	16		276	53.0 ± 3.61	5.21
3	.500	13		272	58.1 ± 2.14	4.68
4	1.000	22		282	70.8 ± 3.80	3.98
5	2.000	11		288	65.5 ± 4.62	4.40
6	4.000	23		279	55.8 ± 3.94	5.00
7	None		27	252	36.3 ± 3.85	6.94
8	.250		18	273	40.6 ± 2.31	6.72
9	.500		12	271	42.2 ± 2.10	6.42
10	1.000		20	275	56.2 ± 2.64	4.89
11	2.000		12	271	51.0 ± 2.92	5.31
12	4.000		24	273	43.7 ± 3.16	6.24

* During 28-day test period. † Stand. error of mean.

weanling rats were depleted of their Vit. A reserve and the depleted animals arranged into comparable groups as in the previous experiment. The rats comprising one-half of the groups were continued on the Vit. A-deficient basal diet whereas the rats of the remaining groups received the basal diet after incorporating cholorotetracycline at the rate of 1 g/kg diet. In addition, the rats comprising the respective experimental groups received measured amounts of beta carotene or Vit. A acetate ranging from 0.0 to 8.3 I.U. daily. These rats were also continued under observation for 28 additional days.

Inasmuch as the rats that received the chlorotetracycline-containing diet increased in body weight faster than did control animals and invariably consumed more food, a third experiment was carried out. Weanling male rats were depleted of their Vit. A reserve as before and arranged into 2 comparable groups. One group received the basal diet and the other received the chlorotetracycline-containing diet. Dietary allowances were restricted so as to equalize the amount of food consumed by each rat during the respective weeks of the 4-week experimental period. All rats received 1.0 μg beta carotene daily during the 28-day test period.

Results. From the results of these studies (Table I) it was evident that growth rates of the depleted rats were most favorably affected by chlorotetracycline concentrations in the diet ranging from 250 to 1000 mg/kg. Optimum concentration of the antibiotic for growth appeared to be approximately 1 g/kg of diet. While this concentration of chlorotetracycline is considerably higher than that usually employed in experimental diets it is to be noted that both male and female rats attained maximum growth at this level. Lower concentrations of the antibiotic were less effective and higher concentrations appeared to exert a depressing effect on growth. On the particular rate of carotene intake (1 μg daily) average increase in rate of growth of male and female rats over that of their respective controls was 66 and 55% respectively.

Since Murray *et al.*(8) reported that the growth rate of rats that received adequate intakes of Vit. A was unaffected by incorporating chlorotetracycline in the experimental diet a series of growth studies was carried out to establish the level of Vit. A or beta carotene intake eliciting the maximum effect of the antibiotic on growth. It may be noted (Tables II, III) that the antibiotic exerted its maximum effect where the test animal received the minimum amount of the vitamin required to maintain body weight. When 0.43 μg of beta carotene was given daily the rats that received the antibiotic-containing diet increased in body weight approximately 3 times as much as control rats. However, when 5.0 μg of the provitamin was given daily there was no marked difference in growth rates of the 2 groups of rats. Growth rates of both male and female rats from 3 different strains were similarly affected by the anti-

TABLE II. Effect of Ingesting a Chlorotetracycline-Containing Diet on Growth Response of Vitamin A-Deficient Rats when Given Varying Amounts of Beta Carotene.

Animal group No.	Added chlorotetracycline, g/kg diet	Carotene supplement, μg/daily	Rats/group ♂	Rats/group ♀	Mean wt of diet consumed,* g	Mean wt gain,* g	Diet consumed/g of gain, g
13	None	.43	8	7	207	8.5 ± 2.14†	24.35
14	1	.43	8	8	252	25.1 ± 2.42	10.04
15	None	.57	14		226	20.2 ± 2.54	11.19
16	1	.57	16		257	36.4 ± 2.67	7.06
17	None	.57		16	215	14.5 ± 1.93	14.83
18	1	.57		16	252	27.4 ± 2.61	9.20
19	None	1.00	20		265	46.6 ± 2.01	5.70
20	1	1.00	20		286	69.8 ± 2.22	4.15
21	None	1.00		14	253	36.3 ± 3.03	6.97
22	1	1.00		14	276	58.5 ± 2.93	4.72
23	None	2.00	6	6	306	56.0 ± 2.92	5.46
24	1	2.00	6	6	327	67.1 ± 2.44	4.97
25	None	4.00	11		321	87.8 ± 3.12	3.66
26	1	4.00	11		343	92.6 ± 2.86	3.70
27	None	4.00		12	322	55.7 ± 3.14	5.78
28	1	4.00		12	320	59.4 ± 2.71	5.39
29	None	5.00	6	4	328	75.2 ± 3.17	4.36
30	1	5.00	6	4	334	76.0 ± 2.96	4.39

* During 28-day test period. † Stand. error of mean.

TABLE III. Effect of Ingesting a Chlorotetracycline-Containing Diet on Growth-Response of Vitamin A-Deficient Rats While Receiving Varying Amounts of Vitamin A Acetate.

Animal group No.	Added chlorotetracycline, g/kg diet	Vit. A acetate supplement, μg/daily	Rats/group ♂	Rats/group ♀	Mean wt of diet consumed,* g	Mean wt gain,* g	Diet consumed/g of gain, g
31	None	.258	15	16	221	15.8 ± 2.39†	14.0
32	1	.258	18	13	247	31.1 ± 2.43	7.9
33	None	.516	22	14	245	31.4 ± 2.91	7.8
34	1	.516	23	13	264	47.5 ± 3.15	5.6
35	None	1.032	6	6	298	64.3 ± 4.11	4.6
36	1	1.032	7	5	327	78.1 ± 3.10	4.2

* During 28-day test period. † Stand. error of mean.

biotic. Comparable intakes of Vit. A acetate and beta carotene were equally effective in promoting growth in presence of the antibiotic.

Invariably, the amount of food ingested per gram of growth was less for the rats that received the chlorotetracycline containing diets. When dietary allowance was restricted but adequate, (Table IV) the rats that received the antibiotic-containing diet increased in body weight approximately 20% more than controls in the same period of time and on the same amount of diet.

Discussion. Diets that contained 50 or 100 mg of chlorotetracycline/kg did not prove uniformly effective in stimulating additional growth in Vit. A-deficient rats that received 1 μg of beta carotene daily. Some of the test animals of the respective groups grew faster than corresponding control animals while others showed no marked difference in growth rate. However, when concentration of the antibiotic was increased to 250 mg or more per kg of diet increases in growth response became more uniform with the result that consistent increases were observed until the antibiotic concentration reached 1 g/kg. A somewhat similar situation was observed where extremely low levels of beta carotene or Vit. A were fed. On the low levels of Vit. A intake some of the test animals died or failed to make gains in body weight during the 28-day period even though the diet contained 1 g of chlorotetracycline/kg. This

TABLE IV. Effect of Ingesting a Chlorotetracycline-Containing Diet on Growth of Vitamin A-Deficient Male Rats Receiving Controlled Food Intakes and 1 μg Beta Carotene Daily over a 28-Day Test Period.

Animal group No.	Added chlorotetracycline, g/kg diet	Rats/group ♂	Mean wt of diet consumed,* g	Mean wt gain,* g	Diet consumed/g of gain, g
37	None	10	250	40.7 ± 2.2†	6.14
38	1.00	10	250	49.9 ± 2.4	5.01

* During 28-day test period. † Stand. error of mean.

made it difficult to evaluate the effect of the antibiotic on growth or on food consumption. However, when Vit. A intake approached 0.7 I.U. daily, growth response became more uniform within each experimental group. Vitamin A intakes inadequate for sustaining life in the control animals over the 4-week test period were effective in sustaining life and often resulted in appreciable growth in rats that received the chlorotetracycline-containing diet. Furthermore, when the antibiotic was incorporated in the basal ration during the depletion period the test animals grew faster than control animals and were somewhat larger when depleted but required no additional time for depletion. Rats transferred to the antibiotic-containing diet after being depleted on the basal diet but given no Vit. A supplement died just as early as control animals subsisting on the basal diet through the entire period. Rats depleted on the antibiotic-containing diet and then transferred to the basal diet performed similarly to animals retained on the basal diet. In general, food consumption paralleled growth rate but in all instances where comparisons were possible it required less of the antibiotic-containing diet to produce a unit of body-weight gain than it did of the basal diet. While this observation has been reported many times a clear-cut explanation of the phenomenon is lacking. No consistent evidence of increased tissue storage of Vit. A was found following chlorotetracycline feeding. However, this could hardly be expected where daily intake of the vitamin was of the range employed in the present studies.

Summary. Addition of chlorotetracycline to a Vit. A-deficient diet was found to stimulate additional growth in Vit. A-deficient rats that received restricted intakes of beta carotene or Vit. A. Extent of additional growth depended on amount of the antibiotic incorporated in the basal diet and amount of the supplementary vitamin fed. Under the conditions of the experiments reported a diet that contained 1 g/kg of chlorotetracycline exerted the greatest growth-stimulating effect in rats that subsisted on a minimum maintenance intake of Vit. A or beta carotene. This increase in growth ranged from 200% in the low Vit. A-intake animals to essentially no increase in growth rate of animals that received the higher doses of the vitamin. Food requirement per unit of weight increase was lower in the rats that received the chlorotetracycline-containing diets. Rats restricted to equal food intakes made greater gains in weight when the diet contained the antibiotic.

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