

## AUREOMYCIN-VITAMIN B<sub>12</sub>

# Influence on Growth, Moisture, Fat, and Nitrogen Content

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The effect of different levels of aureomycin with and without vitamin B<sub>12</sub> on growth and chemical composition of dba mice has been studied. Eighty weanling males and 80 females were divided into lots of 10 and fed the experimental diets for 14 weeks, after which water, fat, and protein body content were determined. The basal diet had the following per cent composition: purified casein, 30; sucrose, 48; lard, 15; salts, 5; and Alphacel, 2. To this mixture the following vitamin supplements were added (per kg.): thiamine hydrochloride, 10 mg.; riboflavin, 10 mg.; pyridoxine, 10 mg.; calcium pantothenate, 40 mg.; choline chloride, 500 mg.; biotin, 200  $\gamma$ ;  $\alpha$ -tocopherol, 40 mg.; vitamin A ester concentrate, 337.5 mg. (67,500 units); and vitamin D, 12.5 mg. (5000 units). To the basal diet were added 25 mg. of aureomycin hydrochloride; 50 mg. of aureomycin hydrochloride; 100 mg. of aureomycin hydrochloride; 5  $\gamma$  of vitamin B<sub>12</sub>; 5  $\gamma$  of vitamin B<sub>12</sub> and 25 mg. of aureomycin hydrochloride; 5  $\gamma$  of vitamin B<sub>12</sub> and 50 mg. of aureomycin hydrochloride; 5  $\gamma$  of vitamin B<sub>12</sub> and 100 mg. of aureomycin hydrochloride. In the male mouse the level of aureomycin had no effect on the average daily weight gain at the end of 5 and 10 weeks on the diet. Addition of vitamin B<sub>12</sub> had a beneficial effect on the average daily gain rate at the end of 5 weeks, but the effect was masked by the end of the tenth week. All levels of aureomycin, with and without vitamin B<sub>12</sub>, caused a decrease in the water and protein content and an increase in the fat content. In the female mouse aureomycin with and without vitamin B<sub>12</sub> had no effect on the average daily weight gain at the end of 5 and 10 weeks on the diet, or on the water, protein, and fat content.

THE GROWTH OF FEMALE MICE was shown by experiments conducted in this laboratory (4) to be improved by supplementing the purified diet containing 23% casein with 100 mg. of aureomycin hydrochloride per kilogram of diet. As Biely and March (2) noted considerable differences in the response of chicks to aureomycin hydrochloride as a result of differences in the composition of the diet fed, it seemed pertinent to investigate the growth response to aureomycin hydrochloride with and without vitamin B<sub>12</sub> of male and female mice fed a diet high in protein. It also appeared that aureomycin hydrochloride might stimulate other responses if fed beyond the growth period. For this purpose moisture, fat, and nitrogen were determined on mature mice which had been fed aureomycin with and

without vitamin B<sub>12</sub> beyond the growth period.

The composition of the basal diet is shown in Table I. To the basal diet were added (per kg.) 25 mg. of aureomycin hydrochloride; 50 mg. of aureomycin hydrochloride; 100 mg. of aureomycin hydrochloride; 5  $\gamma$  of vitamin B<sub>12</sub>; 5  $\gamma$  of vitamin B<sub>12</sub> and 25 mg. of aureomycin hydrochloride; 5  $\gamma$  of vitamin B<sub>12</sub> and 50 mg. of aureomycin hydrochloride; and 5  $\gamma$  of vitamin B<sub>12</sub> and 100 mg. of aureomycin hydrochloride.

Eighty female and 80 male weanling mice of the dba strain were divided into 8 lots of 10 each and fed the experimental diets *ad lib*. The mice were weighed three times per week during the first 10 weeks. At the end of 14 weeks on the experimental regimen, the mice were fasted overnight and sacrificed the

next morning. The gastric and intestinal contents were removed and the animals were cut up in small pieces. The body moisture, fat, and nitrogen content of the mice were determined by the laboratory procedures of the Association of Official Agricultural Chemists (7). The moisture content was determined by drying the animal in a vacuum oven at 90° to 95° C. for 48 hours. The moisture-free samples were then extracted with chloroform in a Soxhlet extractor for 16 hours and dried in the drying oven overnight. The nitrogen content of the moisture- and fat-free samples was determined by the Kjeldahl method. In order to obtain a colorless solution, the samples were digested for 6 hours.

The average daily growth rate of the mice over the 5- and 10-week periods on the experimental diets is summarized

**Table I. Composition of Basal Diet**

Component	%
Purified casein	30
Sucrose	48
Lard	15
Salts	5
Alphacel	2
	Mg./kg.
<b>Vitamin supplements</b>	
Thiamine hydrochloride	10
Riboflavin	10
Pyridoxine	10
Calcium pantothenate	40
Choline chloride	500
Biotin, $\gamma$	200
$\alpha$ -Tocopherol	40
Vitamin A ester concentrate (67,500 units)	337.5
Vitamin D (Viosterol) (5000 units)	12.5

in Table II. The moisture, fat, and nitrogen body content of female and male mice are summarized in Table III.

The addition of aureomycin hydrochloride with and without vitamin B<sub>12</sub> and of vitamin B<sub>12</sub> alone had no beneficial effect on the growth rate of the female mice. Aureomycin hydrochloride per se had no beneficial effect on the growth of male mice. The addition of vitamin B<sub>12</sub> to the diet of the male mice resulted in a growth response during the first 5 weeks of growth, but the rate of growth was not maintained uniformly throughout the 10 weeks. This appears to indicate that in case of the male either vitamin B<sub>12</sub> is not synthesized at a sufficiently rapid rate to meet the demands for optimum growth during the period of very active growth or that the establishment of the intestinal flora requires a longer period of time as compared to female mice. Interestingly, aureomycin hydrochloride at the levels used did not augment or impede the growth-stimulating effect of vitamin B<sub>12</sub>. In a previous study, Mirone (4) reported that the addition of aureomycin hydrochloride to a purified diet containing 23% casein resulted in an increased growth rate of female mice. In this study the addition of aureomycin hydrochloride to a purified diet containing 30% casein did not result in an increased growth rate. This finding is in agreement with the reports of Scott and Glista (6) and Oleson *et al.* (5) that the composition of the diet plays an important role in the growth response of animals to aureomycin hydrochloride. In a recent review Mickelsen (3) stated that in the case of mice the effect of aureomycin hydrochloride appears to be inversely proportional to the nutritional quality of the diet. Doubtless, at least in so far as the mouse is concerned, the quality of the diet plays an important role in the type of growth response to aureomycin.

The results shown in Table III indicate the lack of response of mature females to aureomycin hydrochloride with and without vitamin B<sub>12</sub> and to vitamin

B<sub>12</sub> alone, ascertained by the body moisture, fat, and nitrogen content of the experimental animals as compared to controls. In the male mice there was an increase in body fat with accompanying decrease in moisture and nitrogen with all levels of aureomycin hydrochloride used. The effect was augmented by the addition of vitamin B<sub>12</sub> and the magnitude of increase was greatest when vitamin B<sub>12</sub> alone was fed. This finding again brings out the difference in response to aureomycin hydrochloride and vitamin B<sub>12</sub> of females as compared to males. This difference in response is worthy of further investigation, for it may lead to the elucidation of fundamental differences in the nutritional mechanisms operative in the two sexes. Further work may also indicate a distinct advantage in the simultaneous administration of vitamin B<sub>12</sub> along with aureomycin hydrochloride in the treatment of humans or animals with debilitating diseases, and in cases of extreme emaciation resulting from starvation or inadequate nutrition.

### Conclusions

Aureomycin hydrochloride with and without vitamin B<sub>12</sub> had no effect on the daily growth rate of male and female mice fed a diet containing 30% casein. Vitamin B<sub>12</sub> alone had no effect on the growth of the female, but it enhanced the growth of the male during the first

5 weeks on the experimental diet. Aureomycin hydrochloride with and without vitamin B<sub>12</sub> had no effect on the body moisture, fat, and nitrogen of the female. However, the body fat content of the male was increased, with accompanying decrease in body moisture and nitrogen content.

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**Table II. Average Daily Growth Rate of Mice over 5- and 10-Week Periods**

Diet	Females, Gram		Males, Gram	
	5 weeks	10 weeks	5 weeks	10 weeks
Basal	0.27	0.17	0.34	0.23
Basal + 25 mg. aureomycin HCl	0.26	0.17	0.35	0.24
Basal + 50 mg. aureomycin HCl	0.26	0.17	0.35	0.22
Basal + 100 mg. aureomycin HCl	0.28	0.19	0.35	0.22
Basal + 5 $\gamma$ vitamin B <sub>12</sub>	0.28	0.18	0.38	0.26
Basal + 5 $\gamma$ vitamin B <sub>12</sub> + 25 mg. aureomycin HCl	0.28	0.19	0.38	0.25
Basal + 5 $\gamma$ vitamin B <sub>12</sub> + 50 mg. aureomycin HCl	0.28	0.18	0.38	0.25
Basal + 5 $\gamma$ vitamin B <sub>12</sub> + 100 mg. aureomycin HCl	0.27	0.16	0.38	0.23

**Table III. Moisture, Fat, and Nitrogen Body Content of Mice at End of 14 Weeks**

Diet	Moisture, %	Wet-Weight Basis	
		Fat, %	Nitrogen, %
		<b>Females</b>	
Basal	63.89	12.76	3.18
Basal + 25 mg. aureomycin HCl	63.25	13.19	3.24
Basal + 50 mg. aureomycin HCl	62.65	13.60	3.25
Basal + 100 mg. aureomycin HCl	66.06	11.62	3.03
Basal + 5 $\gamma$ vitamin B <sub>12</sub>	62.78	13.64	3.23
Basal + 5 $\gamma$ vitamin B <sub>12</sub> + 25 mg. aureomycin HCl	63.02	14.13	3.11
Basal + 5 $\gamma$ vitamin B <sub>12</sub> + 50 mg. aureomycin HCl	66.13	11.53	3.08
Basal + 5 $\gamma$ vitamin B <sub>12</sub> + 100 mg. aureomycin HCl	64.27	12.81	3.13
		<b>Males</b>	
Basal	65.07	11.53	3.21
Basal + 25 mg. aureomycin HCl	63.06	13.09	3.24
Basal + 50 mg. aureomycin HCl	59.71	14.87	3.46
Basal + 100 mg. aureomycin HCl	59.65	17.56	3.04
Basal + 5 $\gamma$ vitamin B <sub>12</sub>	57.49	20.52	2.88
Basal + 5 $\gamma$ vitamin B <sub>12</sub> + 25 mg. aureomycin HCl	59.34	17.89	3.04
Basal + 5 $\gamma$ vitamin B <sub>12</sub> + 50 mg. aureomycin HCl	60.06	16.78	3.10
Basal + 5 $\gamma$ vitamin B <sub>12</sub> + 100 mg. aureomycin HCl	61.98	14.95	3.12