Effect of Aureomycin on Utilization of Vitamin A in the Growing Male Albino Rat. (20259)

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Recent investigations by Lih and Baumann (1) and by Sauberlich(2) have shown that antibiotics exert a "sparing effect" on the requirements of vitamins of the "B" complex in the rat. The nature of this "sparing" has not been elucidated, but is generally thought to be due either to an increased synthesis of B vitamins by micro-organisms of the intestinal tract, or to an increased efficiency of absorption of the vitamin from the tract. Burgess *et al.*(3) and also Coates *et al.*(3a) observed in the case of the chick receiving adequate vit. A or precursors plus Na-penicillin G at a level of 30 mg/kg of diet, in addition to significantly increased body weight, a statistically significant ($p = .01$) increase in both total liver vit. A and vit. A per gram of fresh liver.

It was thought that investigations of the effect of an antibiotic (aureomycin) on the vit. A requirement of the growing male albino rat, using growth as a criteria of dietary vit. A adequacy, would be of interest. In addition to ascertaining whether an antibiotic increases liver stores of vit. A in the rat as in the chick, data on kidney stores were also obtained since Johnson and Baumann(4) and Eden and Moore(5) report that on low intakes of vit. A or carotenoids kidney storage of vit. A exceeds liver storage in the rat. It was also thought that this experiment might elucidate somewhat the function of antibiotics. If antibiotics act in growth stimulation by aiding in absorption of a vitamin, partial sparing of the vit. A requirement would be expected, whereas if such function be one of aiding in intestinal synthesis no sparing of vit. A would be anticipated.

Experimental. In an experiment replicated twice male rats were randomly assigned to 6 lots of 5 animals each. They were kept in individual screen bottom cages and given food and water *ad libitum*. The animals were weighed at weekly intervals, while weekly food consumption was determined by weighing back left-over food on the same day the animals were weighed. Both experiments lasted 7 weeks and upon termination vit. A assays were made on the liver and kidneys of each rat in the second replicate by the method of Gallup and Hoefer(6). The basal diet consisted of vitamin-free casein 22%, salt mix 446(7) 4%, cerelose 68.5%, cotton seed oil 5%, complete B vitamin mix* 0.1%, choline (25%) dry mix 0.4%, plus delsterol added to the complete diet at 0.05%. The natural ester form of vitamin A was administered to each rat daily in the form of 0.1 ml of a petroleum ether (skellysolve B) solution of vit. A and D concentrate oil† added to 2 g of the diet in a manner identical to that employed by Koehn(8). The petroleum ether solution contained 1.0 mg α-tocopherol acetate per 0.1 ml of solution. Aureomycin was the antibiotic used. Table I summarizes the treatments given the respective lots. The levels of vit. A ester were chosen so that lots III and IV would be receiving a sub-optimal level for maximum rate of growth, while lots V and VI would be receiving a level above the optimal [Koehn(8)].

TABLE I. Experimental Feeding Plan.

	Additions to vit. A—low basal diet	
Lot No.	Vitamin A, γ/rat/day	Aureomycin, mg/kg diet
1	0	0
2	0	100
3	.75	0
4	.75	100
5	7.5	0
6	7.5	100

* Vit. premix:

Thiamine HCl	500	mg
Riboflavin	600	"
Pyridoxine HCl	300	"
Ca. pantothenate	3	g
Nicotinic acid	6	"
Folic acid	100	mg
Vit. K	100	"
Vit. B_{12}	.5	"
Biotin	10.0	"
Cerelose to make	100	g total

† Distillation Products Industries, Rochester, N. Y.

TABLE II. Average Total Weight Gains and Food Consumption at 49 Days.

Group	A (vit. A-free)		B (0.75 γ vit. A/day)		C (7.5 γ vit. A/day)	
Lot No.	1*	2† (aureomycin)	3	4 (aureomycin)	5	6 (aureomycin)
Wt gain, g	75.6‡	66.9	198.5	180.2	251.6	252.9
Food consumption, g	258 ‡	209	485	487	601	639

* Values attained at time of death (survival time 42.4 ± 3.5 days).
† " " " " " " (" " 29.6 ± 1.8 days).

‡ Significant at $P < 0.05$ where $t = \left[\frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{\Sigma x_1^2 + \Sigma x_2^2}{(N_1-1)+(N_2-1)}}} \times \sqrt{\frac{N_1 N_2}{N_1+N_2}} \right]$

where X = mean value, x = deviation from mean, N = No. of observations.

TABLE III. Vitamin A Content of Liver and Kidneys of Rats from Second Replicate.

Group	A		B		C*	
Lot	1	2 (aureomycin)	3	4 (aureomycin)	5	6 (aureomycin)
Total vit. A/liver, γ	0	0	0-trace	0-trace	75.9 ± 7.48	79.6 ±15.22
Vit. A, γ/g fresh liver	0	0	"	"	7.8 ± 1.22	6.5 ± 1.14
Total vit. A/pair kidneys, γ	0	0	"	"	3.7 ± .54†	1.5§± .37
Vit. A, γ/g fresh kidney	0	0	"	"	2.2 ± .25‡	.9§± .21

* All values are expressed as mean ± stand. error.
† Significant at P = .05. ‡ Significant at P = .01.
§ 4 animals only. All other figures refer to 5 animals.

Results and discussion. Weight gain and food consumption data for the complete experiment are summarized in Table II. In Table III are given the vit. A contents of the liver and kidneys of the rats in the second replicate. Differences between groups A, B and C (Tables II and III) have been tested for statistical significance by comparing the highest mean value in section A (*i.e.*, 1 or 2) with the lowest in section B (3 or 4) etc. All differences were found to be significant at the 0.001 level except food consumption of groups B and C which only reach the 0.01 level.

In the case of both lots I and II the typical syndrome of avitaminosis A was observed, but it was noted that the symptoms appeared earlier in the aureomycin treated animals and seemed to be more severe. This observation is given increased significance when survival times for the lots concerned are compared. Emslie(9) in a personal communication with one of us (B.C.J.), referring to work previously cited(3), indicated that in the chick a vit. A deficiency is produced more rapidly on a vit. A deficient diet plus an antibiotic than on a vit. A deficient diet alone. It appears from Table II that the animals of lot I made greater gains than the animals of lot II due to the greater food intake. Animals receiving sub-optimal or above-optimal levels of vit. A do not demonstrate the effects of increased food consumption or increased weight gains when aureomycin is added to the diet.

The vit. A assay results of livers and kidneys are in reasonably good agreement with the published data of Moore and Sharman (10).

In both lots V and VI liver storage far exceeded kidney storage both on the basis of total content and concentration; comparison here with the previously cited work is not possible because the duration of supplementation is probably critical. It is interesting to note at this point that kidney storage of vit. A in lot 6 was significantly lower than that of lot 5 both as to total amount present and as to concentration in the fresh tissue. An elucidation of this finding must await further investigation.

No vit. A could be detected on analysis of

individual kidneys and livers of rats of lots III and IV as evidenced by the lack of blue coloration with $SbCl_3$ reagent. Petroleum ether extracts of livers and of kidneys from these lots were pooled in order to obtain a greater concentration of vitamin per colorimeter tube. These showed only a very slight blue coloration. From consideration of the data of Moore and Sharman(10) it is evident that very small amounts of vit. A should be present in the tissues concerned at 0.75 γ per day level of supplementation.

It would appear, on the basis of these data, that aureomycin does not function in the absorption of vit. A from the tract. Whether this is true also for the B vitamins requires additional experimental work.

Summary. 1. Growing male albino rats were fed vit. A ester at levels of 0, 0.75, and 7.5 γ/rat/day. Comparisons were made at these levels of vit. A intake with and without aureomycin added to the diet at the rate of 100 mg/kg. 2. Statistically significant differences were found in weight gains and food intakes between groups receiving different levels of vit. A. 3. Statistically significant differences were not found in weight gains and food intakes between groups receiving antibiotic and their controls at the various levels of intake of vit. A, except on vit. A-free diets. Here weight gains and food intake of the control animals were significantly greater than for animals receiving antibiotics. 4. At a level of intake of vit. A of 7.5 γ/day no significant difference of liver storage was found upon aureomycin supplementation. Kidney storage of aureomycin supplemented animals was significantly less than that of control animals. Under conditions of this experiment at the 7.5 γ/day level of supplementation of vit. A significantly greater storage of vit. A occurred in the liver than in the kidneys. 5. No effect of sparing of vit. A by aureomycin was demonstrated, but on vit. A-free diets aureomycin appeared to enhance the deficiency syndrome.

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Acid Phosphatase in Human Female Genital Tract, A Histochemical and Biochemical Study.* (20260)

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In the course of histochemical studies of the normal and malignant human cervix uteri on the possible relationship of alkaline phosphatase activity at pH 7.4 and 9.0, with various phosphorylated derivatives, to glycogen deposition, surprisingly slight activity of the cervical epithelium was a consistent finding.

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