

Relative Growth Responses of Previously Depleted Male and Female Rats to Vit. A Supplementation.* (22448)

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In quantitative estimation of vit. A by biological procedure, it has been considered necessary that tests be conducted in strict parallel with enough experiments to eliminate the effect of individual variability and with rigid control of all factors such as previous nutritional history, weight and sex of experimental animals, litter variability, and rate of growth during the test(1). It is generally believed that test animals from the same litter respond more uniformly than do rats from miscellaneous selection. To obtain reliable results, it is necessary that test animals from different litters be equally distributed among the assay groups. In assigning litter-mate rats to these groups, it is important that sex and body-weight (depleted) be evenly distributed among the groups. There have been differences of opinion, as to the influence of certain of the above factors on outcome of the assay. Sherman and Smith(2) stated, "There is clearly a tendency toward a reverse relationship between body weight of animal and its rate of gain upon a limited allowance of vit. A," "... that among animals of same species and age, the amount of vit. A required to support a given rate of growth tends to vary with size of the individual." However, the influence of sex of test animal on results of assay seems somewhat less definite. According to their findings, the difference in mean weekly growth-rate of 148 male rats compared to a like number of female rats over 8-week test, in which each animal received daily the same but limited quantity of vit. A was 0.3 g (total gain 24.6 and 22.3 g, respectively). As this difference appeared of questionable significance statistically, the authors suggest that. "... for investigations in which an extraordinary degree of refinement

is sought, it might be slightly preferable to work entirely with animals of the same sex." Coward determined standard deviation of response of depleted male and female rats to doses of vit. A administered daily over a 5-week period(3). She found the standard deviation of increase in body weight of male rats to be greater than that of females and the curve of response of the male rat to a dose of vit. A to be greater than that of the female, under comparable experimental conditions. It was her opinion that, if test animals are properly depleted of their body reserve of vit. A, no greater accuracy is obtained through use of isogenic pairs of animals than rats from different litters(4). Other investigators have reported that under certain conditions male rats make greater gain in body weight while receiving a restricted intake of vit. A than do female rats under comparable conditions. Some authors have attributed the difference in growth rate to difference in body weight of the 2 sexes at beginning of the assay. Data obtained during the last few years in the course of vit. A-carotene assays and vit. A storage studies shed further light on the importance of the above mentioned precautions in carrying out biological assays for this vitamin and are presented herewith.

Methods and materials. Young rats ranging from 21 to 24 days of age and between 42 to 48 g in weight were placed in individual all-metal cages having raised screen floors. Each rat was given a liberal but known amount of U.S.P. vit. A-deficient diet(1) and distilled water. The caging, feeding weighing, and care during depletion period and subsequently during supplementary feeding period were carried out essentially as recommended in the U.S.P. technic. Each rat comprising an assay group received for 28 days the same amount of U.S.P. vit. A standard, or of crystalline vitamin or provitamin as a supplement

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TABLE I. Effect of Dosage of Vit. A on Relative Growth Response of Male and Female Rats Previously Depleted of Their Body Reserve of the Vitamin.

Units vit. fed daily*	Males				Females				Ratio of ♂/♀ gain
	No. of rats	Depletion wt (g)	Gain in wt (g) †	Gain/28 units vit. (g)	No. of rats	Depletion wt (g)	Gain in wt (g) †	Gain/28 units vit. (g)	
1.	13	113	26 ± 2.5	26	8	111	28 ± 2.9	28	.93
1.07	96	110	32 ± 2.7	30	78	109	34 ± 2.4	32	.91
1.4	22	117	40 ± 2.4	29	10	115	39 ± 3.1	28	1.03
1.5	210	115	46 ± 4.9	31	174	114	42 ± 5.0	28	1.09
1.93	30	119	62 ± 3.6	32	27	116	53 ± 4.2	28	1.17
2.0	71	114	67 ± 5.8	34	69	110	55 ± 4.6	27	1.22
2.9	19	113	79 ± 5.2	27	11	111	64 ± 3.8	22	1.23
5.7	8	121	86 ± 4.9	15	3	119	65 ± 3.1	12	1.32
10.	7	116	80 ± 6.9	8	4	113	62 ± 5.9	6	1.29
18.	8	107	80 ± 5.4	4	6	107	66 ± 3.6	4	1.21
25.	21	107	84 ± 4.3	3	6	102	62 ± 6.3	2	1.35
36.	8	107	82 ± 6.7	2	6	105	54 ± 6.7	2	1.52
40.	3	104	103 ± 3.2	3	3	101	70 ± 4.3	2	1.47
50.	23	102	83 ± 7.4	2	5	107	65 ± 5.1	1	1.28
70.	48	104	99 ± 6.2	1	24	105	71 ± 6.5	1	1.39

* U.S.P. units or calculated equivalent.

† Mean wt with stand. error.

to the deficient diet. These supplements were dispensed in a purified cottonseed oil medium which was of such concentration as would limit the amount of solution fed to each rat to 0.7 ml per week. This was administered as 2/7 of the weekly allotment on Monday, 2/7 on Wednesday, and 3/7 on Friday. The calculated daily dosage of vitamin or provitamin ranged from 1.0 to 2.9 U.S.P. units in the assay studies and as high as 70 U.S.P. units/day during vitamin storage studies. During the 4-week test period a record was made of food consumption, body-weight changes, and general appearance of the animals. Data pertaining to mean depletion weight of several groups of animals and to mean growth response during the test period have been compiled and arranged according to sex and increasing daily dosage of the vitamin and are presented in Table I. In addition, typical data reflecting the relative weekly growth response of male and female rats to 4 different dosages of vitamin are presented in Fig. 1.

Results. Data in Table I reveal that there may be a measurable difference in growth response of previously depleted male and female rats to a supplementary allowance of vit. A. This difference was insignificant when daily dosage ranged 1.0 to 1.5 U.S.P. units or in the range generally employed in biological assay for this vitamin. There was some evidence that the female rat makes the greatest

growth response to vit. A supplementation when the daily allowance of vitamin is 1 U.S.P. unit or less. When less than 1 unit was employed, it was usually difficult to evaluate, owing to wide variation in responses of test animals and to other complications. When daily dose of the vitamin ranged from 2 U.S.P. units upward, the male rats grew more than did the females. This difference in growth rate associated with sex was observed consistently and appeared to increase at a gradual rate until daily dosage of the vitamin reached 36 to 40 units. That this difference in growth rate was due to differences in body weights of male and female rats at the beginning of the test period seems untenable from the data at hand. The optimal rate of growth for male rats was 21 to 25 g per week and that for female rats 16 to 18 g. That this difference in growth is not due to differences in sexual development during the period of supplementary feeding is suggested by the data presented in Fig. 1. Here, it is to be noted that, while male rats receiving the higher rates of vitamin supplementation grew at a more rapid rate than did the corresponding females, there was no consistent evidence that either the male rats increased their rate of growth or that the female rats decreased their rate of growth during the 28-day test period. Food intake records indicate a direct relationship between food intake and growth

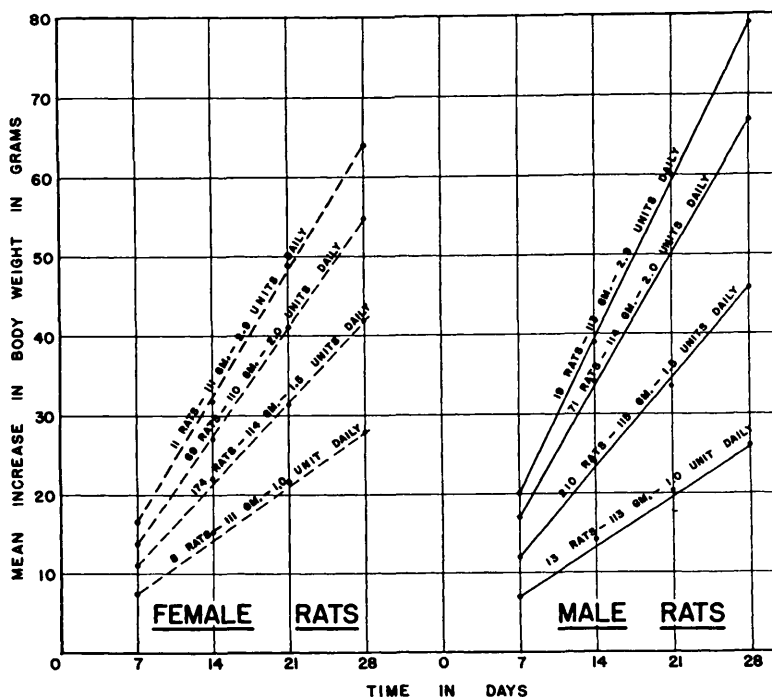


FIG. 1. Relative growth response of male and female rats to 4 different dosages of vit. A administered over a 28-day period.

in both sexes.

Another observation from data in Table I is the relatively similar growth response of both male and female rats to vit. A supplementation within dosage range of 1 to 2 U.S.P. units vit. A daily. This suggests that within this range growth response is a linear function of the vitamin dose. These data also suggest the precaution that should be exercised in interpreting data obtained while attempting to evaluate the relative effectiveness of beta carotene and vit. A in promoting growth in vit. A deficient rats when the carotene is fed at $1\text{ }\mu\text{g}$ (1.67 International units by definition) per day and vit. A at $1\text{ }\mu\text{g}$ (3.33 U.S.P. or International units) per day, or at any dosage range where either of these supplements produce a growth response out of proportion to the dosage.

Summary. Male rats which have been depleted of their body reserve of vit. A grow more during a 28-day test period than do cor-

responding female rats when a daily dosage of 2.0 U.S.P. units or more of the vitamin is fed as the supplement. This increased growth is not directly related to the body weight of the test animal at the beginning of the supplementary feeding period. While it appears that the greater growth capacity is an inherent characteristic of the male rat which is present at the beginning of the test period, it is not clear as to why this characteristic does not manifest itself at all levels of vit. A intake.

1. *Pharmacopoeia of the United States*, Fourteenth Revision, Mack Publishing Co., Easton, Pa., 1950, 787.

2. Sherman, H. C., and Smith, S. L., *The Vitamins*, The Chemical Catalog Co., Inc., 419 Fourth Ave., New York, 1931, 262.

3. Coward, Katherine H., *Biochem. J.*, 1932, v26, 691.

4. *The Biological Standardization of the Vitamins*, William Wood and Co., Baltimore, Md., 1938, 30.

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