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INTRODUCTION

A number of physiological functions in mammals have been reported to be affected by vitamin A deficiency. With the exception of vision, the molecular role of vitamin A in these disturbances has not been ascertained (23). In the majority of studies dealing with hypovitaminosis A, researchers have employed an acute model in which a vitamin A-free diet is fed to weanling rats with a plateau in growth or loss in body weight utilized as the deficiency criteria (6, 17). An alternative is the chronic model in which graded levels of the vitamin are added to a vitamin A-free diet and fed to the weanling rats (6) with one or more criteria, such as, decreased growth rate, elevated cerebrospinal fluid pressure or increased hematocrit, utilized as the criteria of deficiency. Most recently, acute hypovitaminosis A has been produced by feeding a vitamin A-free diet to weanling rats to which retinoic acid was cyclically added to provide synchronous occurrence of the acute deficiency (17). The occurrence, in terms of the number of days fed no retinoic acid, of a number of physiological functions and clinical signs has subsequently been reported (3).

Since unequal feed consumption between deficient and control rats possibly confounded any results due to deficiency of the vitamin, pair-feeding has been employed by some researchers to eliminate differences in feed consumption (17). However, in the acute model, pair-feeding has generally resulted in semi-starvation of the controls due to a marked reduction of appetite in the deficient animals. In the chronic model, elimination or minimization of differences in feed consumption might be possible by employment of equalized feeding (equalized ration offered) (9, 11, 25). In addition a marked reduction in growth would be avoided so that deficiency changes occur relatively rapidly (6).

The present studies were conducted to develop a model of chronic hypovitaminosis A in the weanling rae utilizing equalized feeding. Initially an acute hypovitaminosis A study was conducted so as to determine, in this species, the degree of partial vitamin A depletion with little effect on feed consumption and growth and where, the incidence of characteristic deficiency changes, elevated cerebrospinal fluid pressure and squamous metaplasia of the nasolacrimal duct, were negligible. This was followed by a chronic hypovitaminosis A study in which graded levels of the vitamin were fed under conditions of ad libitum feeding and prior partial vitamin A depletion. Lastly, based on this chronic experiment and subsequent data collected, three chronic vitamin A studies were conducted under conditions of equalized feeding after partial vitamin A depletion.

METHODS

Animals. Weanling 21-day old male Sprague-Dawley albino rats from one source[1] were used in all experiments. Each rat was housed in a stainless steel, wire bottomed, cage[2] in a temperature controlled environment. Daily minimum and maximum ambient temperatures with their respective standard errors averaged 20 ± 2, 22 ± 2, experiment I; 21 ± 1, 23 ± 1 experiment II; 19 ± 1, 23 ± 1 experiment III; 19 ± 1, 21 ± 1 experiment IV and 20 ± 2, 22 ± 1 experiment V. Incandescent light was provided from 7 A.M. to 7 P.M. daily and the amount of light in the animal room, measured every four weeks, averaged 221 ± 15, 206 ± 28, 264 ± 11, 248 ± 6, and 220 ± 5 lux for experiments I through V, respectively.

The composition of the vitamin A-free basal ration, Table 1, was essentially that of Wolf et al. (29) with the mineral mixture that of Williams and Briggs (28). Where necessary, the amounts of ingredients[3] including minerals[4] and vitamins[3] were adjusted to meet the National Research Council minimum daily requirement for the rat as outlined in 1962 (19) for experiments I and II and as outlined in 1972 (20) for experiments III-V. The vitamin A supplement was retinyl acetate[5] but cited as retinol equivalents. Water was provided ad libitum.

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1 Holtzman, Madison, Wisconsin.
2 Hoeltge, HB-26 stainless steel 17.8 x 17.8 x 24.1 cm. Cincinnati, Ohio.
3 General Biochemicals, Chagrin Falls, Ohio.
4 J. T. Baker Co., Phillipsburg, New Jersey.
5 Hoffmann-La Roche, Dry Vitamin Acetate Beadlets, type 325-40, guaranteed to contain, 111,800 μg of retinyl acetate per gram, Nutley, New Jersey.

In the initial study, experiment I-acute hypovitaminosis A, four groups of 20, 24, 30 and 58 rats were obtained. Each rat was fed ad libitum for 7 days the basal ration to which vitamin A acetate equivalent to 0.1 µg retinol/g ration had been added, standardizing period. Based upon random allotment, each rat was then fed ad libitum either the basal ration without added vitamin A, deficient, or the basal ration to which vitamin A acetate equivalent to 1.0 µg retinol/g ration was added, controls, comparison period. Rats were sacrificed at consecutive 7-day intervals, with assignment at random, during this comparison period.

In experiment II-chronic hypovitaminosis A, one group of 16 rats was obtained. Each rat was fed ad libitum the basal ration which contained vitamin A acetate equivalent to 0.1 µg retinol/g ration for 7-days, standardizing period. For the next 21-days each rat received ad libitum the basal ration, partial vitamin A depletion period, the duration of which was based upon experiment I. A 6-week comparison period followed during which each rat received, based upon random allotment, one of four intakes of vitamin A acetate equivalent to 4, 16, 64, or 256 µg retinol/kg body weight/day. At 11 a.m. daily, rats were fed the basal ration containing either 0.4, 1.6, 6.4 or 25.6 µg retinol equivalent/g of ration at 1% of their respective current weekly body weight. When each rat had consumed this initial supplement, usually between 2 and 3 p.m., the rats were fed sufficient basal ration without added vitamin A to provide a weighback, feed refusal, the following morning at 8 a.m. To ascertain vitamin A status of these rats at the completion of the partial vitamin A depletion period, a blood sample from the tail was obtained from 2 of the rats on each treatment. Average plasma vitamin A for these 8 rats was 5.9 µg/100 ml with a standard deviation of 2.8.

In experiment III-chronic hypovitaminosis A, sets of 11 rats each were

obtained weekly over a 9-week period. To monitor vitamin A status, three rats in each set of 11 were sacrificed upon arrival, one at the completion of the 1-week standardizing period and two at the completion of the 3-week partial vitamin A depletion period. Average plasma vitamin A concentrations, μg/100 ml, with standard deviations upon arrival, completion of standardization and completion of partial vitamin A depletion were respectively, 16,4; 24,8 and 8,3. Similar values for average liver vitamin A concentrations expressed as $\log_{10}$ (μg/g 10^2) were 3.38, 0.09; 2.92, 0.16 and 0.98, 0.45. Upon completion of the 3-week partial vitamin A depletion period, the remaining 5 rats of each set of 11 were fed for a 5-week comparison period one of five intakes of vitamin A acetate equivalent to 4, 12, 16, 64 or 256 μg retinol/kg body weight/day. The procedures were identical to those of experiment II except that the allowance of basal ration (amount of the basal ration with vitamin A plus amount of basal ration without vitamin A) was based upon equalized feeding which was derived from body weight and average feed consumption data from all rats of experiment II. The allowance for the first week comparison period was 0.071 times the anticipated body weight, for the second week 0.066, and for the next three weeks, respectively, 0.055, 0.045 and 0.043. The anticipated body weight was calculated as follows:

$$(W_o) + [(W_o - W_{-2}) /4]$$

where W_o = the current weekly body weight and W_{-2} = the body weight 2 weeks prior to W_0.

In experiment IV-chronic hypovitaminosis A, sets of 12 rats each were obtained weekly over a 9-week period. As in experiment-III, three rats were sacrificed upon arrival, one at the completion of the 1-week standardizing period and two after 3-weeks of partial vitamin A depletion. Average plasma vitamin A concentrations, μg/100 ml, with standard deviations were upon

arrival 17, 2, standardization 21,6 and partial vitamin A depletion, 7,3. Similar statistics for liver vitamin A concentrations, $\log_{10}(\mu g/g\ 10^2)$, were 3.37, 0.08; 2.95, 0.16 and 0.51, and 0.60. Upon completion of partial vitamin A depletion, the remaining 6 rats of each set of 12 were fed, according to random allotment, for a 5-week comparison period, one of six intakes of vitamin A acetate equivalent to 8, 12, 16, 20, 64 or 256 µg retinol/kg body weight/day. The allowance of basal ration was increased slightly above that used in experiment III and was, respectively, 0.074, 0.070, 0.059, 0.050 and 0.048 of the of the anticipated body weight for the first through the fifth weeks of the comparison period.

In the last study, experiment V-chronic hypovitaminosis A, sets of 7 rats each were obtained weekly over an 11 week period. To ascertain vitamin A status, 1 rat in each set of 7 was sacrificed upon arrival, 1 after standardization and 1 upon completion of vitamin A depletion. Average plasma vitamin A concentration, in µg/100 ml, with standard deviations, were respectively, 19, 7; 18, 7 and 8, 3. Similar values for liver vitamin A concentrations, in $\log_{10}$ $(\mu g/g\ 10^2)$, were 3.40, 0.08; 3.07, 0.32 and 1.15, 0.26. Upon completion of vitamin A depletion, each rat received daily according to random allotment a deficient or an adequate intake of vitamin A equivalent to 12 or 256 µg retinol/kg body weight for either a 6 or 8 week comparison period. In this experiment, the basal ration allowance during the comparison period was based on the average growth obtained in experiment IV and Hartsook and Mitchell's (13) estimate of daily feed consumption and body weight. Body weight, W, of all rats in experiment IV as a function of days of age, A, equalled:

$$W = 42 + 4.842\ A - 0.025A^2$$

From this equation, the average weekly gains in body weight were computed. The latter values multiplied by ½ were added successively to each rat's body

weight observed at the start of the comparison period to provide anticipated body weights for each of the six or eight comparison periods weeks. Feed consumption, FC, was then computed from these six or eight anticipated body weights, W, utilizing the Hartsook and Mitchell formula (13):

$$FC = 1.4 + 0.120W - 0.000,201W^2$$

The use of an overall average weekly increase in body weight instead of each rat's contemporary body weight in computing anticipated body weight was judged necessary since the latter resulted in feed offered being considerably less in those rats fed the lower vitamin A intakes in experiments III and IV. Thus the objective of equalized feeding was not met in these two experiments, except at intakes greater than 8 µg of retinol equivalent/kg body weight/day.

Observations and analyses. All ration offered and refused was weighed to the nearest 1 g daily. The retinyl acetate supplement was analyzed as described previously (12) and contained the equivalent of 109, 144 µg of retinol/g. Each rat was weighed to the nearest 1 g upon arrival and at successive weekly intervals and at sacrifice.

On the designated sacrifice day, weekly intervals for experiment I-acute hypovitaminosis A, and on the day following the completion of the comparison period, experiments II through V-chronic hypovitaminosis A, each rat was anesthetized intraperitoneally with 45 mg pentobarbital sodium[6] per kilogram of body weight. It was then placed in a prone position on a level wooden platform with the head held at a 20° angle by inserting earbars of a stereotaxic apparatus[7] into the auditory canals. A 25 gauge needle, with the

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[6] Fort Dodge, M-122, 65 mg pentobarbital sodium per milliliter, Fort Dodge, Iowa.

[7] David Kopf, No. 900. Small animal stereotaxic instrument, Tujunja, California.

hub removed and connected via a 30 cm length of 20 gauge polyethylene tubing to a transducer[8] (volume displacement = 0.01 mm^3 Hg pressure) whose diaphram was at the same level with the midline of the ear bars which in turn was connected to a reservoir containing physiological saline (0.85% NaCl), was inserted into the cisterna magna (14). The transmitted cerebrospinal fluid pressure was recorded on a polygraph[9] which had been previously calibrated for each set of rats for hydrostatic pressures corresponding to different heights of the reservoir containing physiological saline by moving the reservoir along a calibrated metric stick.

Upon completion of the above cerebrospinal fluid pressure measurement, a citrated blood sample was obtained by heart puncture, the blood centrifuged to obtain plasma, and vitamin A concentration determined on the plasma by the Neeld and Pearson trifluoroacetic acid micro-method (21). The liver was removed, weighed, macerated and vitamin A determined on an aliquot by a modification of the Gallup-Hoeffer procedure (5). The 2nd lumbar vertebrate of the rats of experiment IV and V was removed and subsequently freed of adhering tissue which included immersion in boiling H_2O for 5 minutes followed by air drying. The total volume and canal volume of this vertebrate was estimated by displacement following the procedures of Gallina et al. (11).

The degree of squamous metaplasia of the nasolacrimal ducts of the rat was assessed in experiments I through IV. After removing the head at the occipital bone, the right and left mandibles were removed along with all skin and adhering musculature and placed in 10% formalin. After decalcification

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[8] Statham, Model P23 GbC, Puerto Rico.
[9] Grass Polygraph 7, Quincy, Massachusetts.

of the heads for 24 hours, 5 transverse sections were taken of the nasal cavity, 3 mm apart, from the snout to the eye. Thin sections were taken from the cut side nearest the eye and stained with haematoxylin and eosin. Each section was studied under light microscopy for metaplastic changes in the epithelial lining of the ducts which is indicative of the vitamin A status of the rat (16).

The data were subjected to analysis of variance (26) to determine variation due to treatment, no vitamin or plus vitamin A at designated sacrifice weekly periods, experiment I-acute hypovitaminosis A, or intakes of vitamin A, experiment II through V-chronic hypovitaminosis A and due to rats within treatment, experimental error. Where appropriate response criterion were adjusted for initial measurements, e.g. body weight gain during the comparison period adjusted for body weight observed at the start of this period, by covariance (26). Linear, quadratic or Norton's bent-line regression (10) were utilized to estimate the rate of change of criteria with time for experiment I-acute hypovitaminosis A and with $\log_{10}$ of vitamin A intake (2) for experiments II through IV-chronic hypovitaminosis A.

TABLE 1. Basal Ration Fed to Rats in Experiments I and II.

Ingredients	kg/100 kg
Casein, vitamin free test	24.5
Dextrin, white	66.5
Cottonseed oil	3.5
Mineral Mix[1]	3.5
Vitamin Premix[2]	1.0
Choline Premix[3]	1.0
	100.0 kg

[1] Contains per kg. of ration: 7.25000 g. $CaCO_3$; 11.30000 g. $CaHPO_4$; 6.51000 g. Na_2HPO_4; 7.29300 g. KCl; 0.00070 g. KIO_3; 2.30000 g. $MgSO_4$; 0.01540 g. $MnSO_4$; 0.15750 g. $FeC_6H_5O_7.xH_2O$ (16% Fe); 0.02200 g. $ZnCO_3$; and 0.01300 g. $CuSO_4$.

[2] Contains: 250 mg. thiamine-HCl; 240 mg pyridoxine-HCl; 1600 mg Ca-pantothenate; 300 mg riboflavin; 100 mg biotin; 40 mg folic acid; 1000 mg inositol; 120 mg p-aminobenzoic acid (PABA); 3000 mg nicotinic acid; 1 mg vit. B_{12}; 100 mg menadione (vit. K_3); 10 mg (100,000 IU) 7-dehydrocholesterol (vit. D_3); and 20,000 mg (3500 IU) 50% dl-α-tocopheryl acetate plus 973.2390 g. cerelose.

[3] Contains 150 g. choline chloride and 850 g. cerelose.

TABLE 1 continued. Basal Ration Fed to Rats in Experiments III, IV and V.

Ingredients	kg/100 kg
Casein, vitamin free test	24.5
Dextrin, white	66.5
Cottonseed Oil	3.5
Mineral Mix[1]	3.5
Vitamin Premix[2]	1.0
Choline Premix[3]	1.0
	100.0 kg

[1] Contains per kg. of ration: 7.24990 g. $CaCO_3$; 11.29975 g. $CaHPO_4$; 6.51000 g. Na_2HPO_4; 7.23030 g. KCL; 0.00070 g. KIO_3; 2.29985 g. $MgSO_4$; 0.15400 g. $MnSO_4$; 0.22050 g. $FeC_6H_5O_7.xH_2O$(16% Fe); 0.02205 g. $ZnCO_3$; and 0.01295 g. $CuSO_4$.

[2] Contains: 250 mg. thiamine-HCL; 1400 mg. pyridoxine-HCL; 1600 mg Ca-pantothenate; 500 mg riboflavin; 100 mg biotin; 50 mg folic acid; 10,000 mg inositol; 1000 mg p-aminobenzoic acid (PABA); 3000 mg nicotinic acid; 1 mg vit. B_{12}; 10 mg menadione (vit. K_3); 2.5 mg (100,000 IU) 7-dehydrocholesterol (vit. D_3); and 7000 mg (3500 IU) 50% dl-α-tocopheryl acetate plus 975.0865 g cerelose.

[3] Contains 150 g choline chloride and 850 g cerelose.

RESULTS

Experiment I-acute hypovitaminosis A. Feed consumption and growth statistics are presented in Table 2. Feed consumption adjusted for body weight at the start of comparison period in g/day, Y_1, as a function of comparison period days, X, was for the rats fed only the basal ration, deficient:

$$Y_1 = 7.7 + 0.499\ X - 0.008X^2 \pm 1.9$$

and for the rats fed the basal ration plus vitamin A, controls:

$$Y_1 = 10.2 + 0.197\ X \pm 1.9$$

Similar functions for $\log_{10}$ terminal body weight adjusted for initial body weight in g, Y_2, and for body weight gain in g/day, Y_3 were:

$$Y_2 = 1.90 + 0.0279\ X - 0.0004X^2 \pm 0.05, \text{ deficient}$$

$$Y_2 = 1.35 + 0.0242X - 0.0002X^2 \pm 0.05, \text{ control}$$

and

$$Y_3 = 4.3 + 0.152X - 0.004X^2 \pm 1.0, \text{ deficient}$$

$$Y_3 = 5.8 \pm 1.0, \text{ control}$$

Feed consumption of the deficient animals was estimated to reach a maximum on the 31st day, terminal body weight at 35 days and gain in body weight at the 19th day. In contrast, the control rats increased their feed consumption at a constant rate throughout the comparison period, $\log_{10}$ terminal body weight continued to increase throughont the comparison period and gain in body weight remained constant.

Plasma and liver vitamin A concentrations are presented in Table 3. Plasma vitamin A concentration, Y_4, decreased during the comparison period in those rats fed no vitamin A but maintained an estimated constant concentration in those rats fed vitamin A as follows:

$$Y_4 = 20-0.54\ X, \text{ deficient.}$$

$$Y_4 = 29 \pm 7, \text{ controls.}$$

In the case of the deficient rats, the rate of decrease of plasma vitamin A of 0.5 µg/100 ml/day, represents an unweighted regression which did not account for the heterogeneity of variance of this criterion whose variance was related to magnitude of the average concentration and thus decreased as days of the comparison period increased. Liver vitamin A concentrations expressed as $\log_{10}$ µg/g 10^2, Y_5, as functions of comparison period days were:

$$Y_5 = 3.03 - 0.1306X + 0.0015\ X^2 \pm 0.36, \text{ deficient.}$$

$$Y_5 = 2.85 - 0.0195X \pm 0.36, \text{ controls.}$$

The deficient rats' liver vitamin A concentration decreased 93% during the 1st 10 days, 86% during the next 10 days and 72% from the 20th through the 30th days whereas in the controls, this decrease was estimated to be constant and amounted to 35% for each 10 days. Plasma vitamin A concentrations, Y_4, according to intervals of 0.30 $\log_{10}$ liver vitamin A concentration, Y_5, are presented in Table 4 (1). In neither the deficient nor control rats did there exist a simple linear relationship between these two variables when the entire range of values were considered. In addition, the deficient rats' plasma vitamin A concentrations at comparable $\log_{10}$ liver vitamin A concentrations to the controls were lower.

Cerebrospinal fluid pressures of the deficient rats, Figure 1, increased 33% for each 7-day increase in the comparison period. In contrast, the pressures of the control rats were unaffected, equalling a geometric mean of 71 mm of saline throughout the comparison period. By placing 95% confidence limits about the statistics for both groups of rats, it was found that the deficient rats' pressure was significantly greater than the controls at 21 days, 28 days and 35 days.

The incidence of nasolacrimal duct squamous metaplasia of the deficient rats, Figure 1, was unaffected until the 20th day of the comparison period, but thereafter the incidence increased markedly. The incidence of squamous metaplasia of the control rats remained constant during the 35 day comparison period.

To establish the degree of association between elevated cerebrospinal fluid pressure or the incidence of squamous metaplasia of the nasolacrimal duct and the vitamin A status of the rat as indicated by plasma and $\log_{10}$ liver vitamin A concentrations (8), data from both the deficient and control rats were grouped according to intervals of plasma vitamin A = 4.0 µg/100 ml, Tables 5 and 6, and liver vitamin A = $\log_{10}$ 0.30 µg/g 10^2, Tables 7 and 8. Cerebrospinal fluid pressure, $\log_{10}$ mm of saline, Y_6, and the incidence of squamous metaplasia of the nasolacrimal duct, arcsin $\sqrt{\%\ \text{positive}}$, Y_7, were found to be related to plasma vitamin A, Y_4 as follows:

$$Y_6 = 1.83 - 0.0725\ (Y_4 - 7.7)\Theta \pm 0.14,\ R^2 = 0.93$$

in which $\Theta = 1$ when $Y_4 \leq 7.7$ and $\Theta = 0$ when $Y_4 > 7.7$

and $$Y_7 = 19.4 - 9.3643\ (Y_4 - 8.1)\Theta \pm 21.8,\ R^2 = 0.92$$

in which $\Theta = 1$ when $Y_4 \leq 8.1$ and $\Theta = 0$ when $Y_4 > 8.1$.

Above plasma vitamin A concentrations of 7.7 µg, the cerebrospinal fluid pressure was maintained at a geometric mean equal to 68 mm of saline and for each 1 µg/100 ml decrease in plasma vitamin A below 7.7, the pressure increased by 15%. In the case of the incidence of squamous metaplasia, above plasma vitamin A concentrations of 8.1, the incidence averaged 19.4 for the arcsin $\sqrt{\%}$ equivalent to 11%, and at 7, 5 and 3 plasma vitamin A concentrations, the incidence was equivalent to 24%, 56% and 85% respectively. The association of these two response criteria and liver vitamin A, $\log_{10}$, µg/g 10^2, Y_5, were as follows:

$$Y_6 = 1.77-0.1909\ (Y_5-2.545)\theta \pm 0.18,\ R^2 = 0.92$$

in which $\theta = 1$ when $Y_5 \leq 2.545$ and $\theta = 0$ when $Y_5 > 2.545$

and $$Y_7 = 16.0 - 60.7624\ (Y_5-1.34)\theta \pm 49.0, R^2 = 0.75$$

in which $\theta = 1$ when $Y_5 \leq 1.34$ and $\theta = 0$ when $Y_5 > 1.34$.

Above liver vitamin A concentrations of 3.51 µg(antilog of 2.545×10^{-2}) cerebrospinal fluid pressure was maintained at a geometric mean of 59 mm of saline (antilog of 1.77), but at concentrations $\leq$ 3.51 µg, each 10% decrease in concentration resulted in a 1.7% increase in the pressure. The incidence of squamous metaplasia of the nasolacrimal duct averaged 7.7% (equivalent to an arcsin $\sqrt{\%}$ of 16) at liver vitamin A concentrations greater than 0.22 µg (antilog of 1.34×10^{-2}). Below this concentration the incidence increased and equalled 19%, 36% and 68% at liver vitamin A concentrations of 0.15, 0.10 and 0.05, respectively.

TABLE 2. Effect of increasing duration of vitamin A deficiency upon feed consumption and body weight gain in the weanling male rat.

Criteria	Vitamin A status	Depletion time, days 0	7	14	21	28	35	SD per rat
Animals, no	-[a]	8	11	5	9	10	11	---
	+[a]	9	9	10	9	10	9	
Feed, g/d								
Offered	-	--	13.6	15.1	16.7	17.8	17.4	1.5
	+	--	13.0	15.0	16.4	17.5	19.2	
Consumed								
Actual	-	--	10.9	13.1	14.7	15.8	14.9	1.9
	+	--	11.2	13.3	14.5	15.2	17.6	
Adjusted[b]	-	--	11.1	12.4	14.8	15.8	15.3	1.8
	+	--	11.5	13.1	14.3	15.0	17.5	
Body weight								
Initial, g[c]	-	--	71	85	73	74	68	10
	+	--	70	76	77	76	76	
Terminal								
Actual	-	74	107	162	191	231	232	68
	+	79	112	158	194	236	279	
Log_{10}	-	1.87	2.02	2.20	2.28	2.36	2.36	0.05
	+	1.89	2.05	2.20	2.28	2.37	2.44	
Log_{10} adjusted[b]	-	1.87	2.03	2.18	2.28	2.36	2.38	0.05
	+	1.88	2.06	2.19	2.28	2.36	2.44	
Gain, g/d	-	--	5.2	5.5	5.6	5.6	4.7	1.0
	+	--	6.0	5.8	5.6	5.7	5.8	

[a] - indicates no dietary vitamin A; + indicates dietary vitamin A fed as retinyl acetate equivalent to 1 µg retinol per gram of basal ration.

[b] Adjusted for initial body weight at the commencement of the comparison period.

[c] Initial body weight at commencement of the comparison period.

TABLE 3. Effect of increasing duration of vitamin A deficiency upon plasma and liver vitamin A concentration in the weanling male rat.

Depletion period, days	Animals, no.		Plasma vitamin A, µg/100 ml		Liver vitamin A, µg/g			
					Actual		Log_{10} [b]	
	−[a]	+[a]	−	+	−	+	−	+
0	6	6	16(9)[c]	26	9.54	6.67	2.96	2.81
2	2	2	21(1)	24	5.67	9.27	2.75	2.94
7	6	6	17(2)	30	1.60	4.40	2.18	2.58
9	5	3	23(6)	31	2.94	9.14	2.40	2.94
14	4	8	16(8)	29	0.37	3.01	1.40	2.43
16	1	2	9(-)	35	0.09	9.49	0.95	2.97
21	5	7	6(2)	28	0.08	3.14	0.80	2.34
23	4	2	7(4)	33	0.11	11.88	0.96	3.08
28	7	7	3(1)	25	0.04	2.57	0.51	2.14
30	3	3	3(1)	38	0.03	10.71	0.48	3.03
35	11	9	3(2)	30	0.02	3.52	0.34	2.01
SD per rat	--	--	--	7	----	----	0.36	

[a] − indicates no dietary vitamin A, + indicates dietary vitamin A fed as 1 µg retinol equivalent per gram of basal ration.

[b] Actual liver vitamin A times 10^2.

[c] SD per rat.

TABLE 4. Plasma vitamin A concentration according to $\log_{10}$ liver vitamin A concentration intervals.

	Deficient		Control	
$\log_{10}$ liver vitamin A interval	Number of rats	Plasma vitamin A	Number of rats	Plasma vitamin A
($\mu g/g\ 10^2$)		($\mu g/100$ ml)		($\mu g/100$ ml)
0.0-0.29	7	4(2-8)[a]		
0.3-0.59	9	3(1-5)		
0.6-0.89	10	3(2-5)		
0.9-1.19	4	8(6-11)		
1.2-1.49	3	12(8-17)	2	26(24-28)[a]
1.5-1.79	1	23	7	25(18-30)
1.8-2.09	4	16(14-17)	5	18(21-24)
2.1-2.39	5	19(16-25)	5	30(23-35)
2.4-2.69	2	24(18-30)	9	32(24-46)
2.7-2.99	7	22(12-28)	17	29(14-38)
3.0-3.29	2	7(5-10)	10	33(18-49)

[a] Range

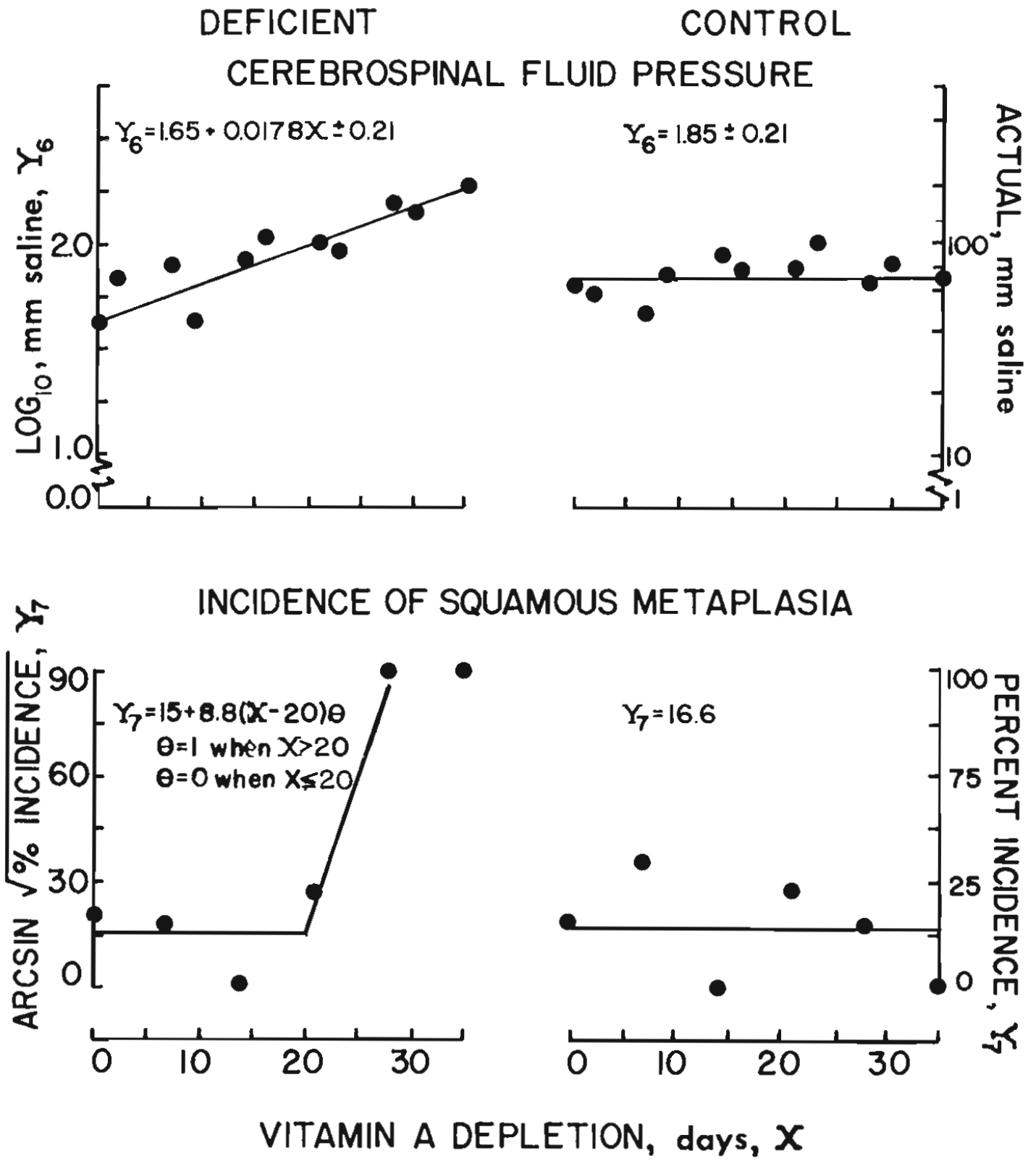

Figure 1. Relationships of $\log_{10}$ cerebrospinal fluid pressure, and incidence of squamous metaplasia of the nasolacrimal duct to duration of vitamin A depletion time for weanling rats fed a vitamin A deficient or vitamin A adequate diet.

TABLE 5. Cerebrospinal fluid pressure according to plasma vitamin A concentration intervals.

Plasma vitamin A interval	Cerebrospinal fluid pressure					
	Deficient		Control		Combined[a]	
	Number of rats	Average	Number of rats	Average	Number of rats	Average
(μg/100 ml)		($\log_{10}$ mm saline)		($\log_{10}$ mm saline)		($\log_{10}$ mm saline)
0-3.9	19	2.25			19	2.25
4-7.9	10	1.96			10	1.96
8-11.9	5	1.78			5	1.78
12-15.9	2	1.82	1	1.70	3	1.78
16-19.9	9	1.90	2	1.86	11	1.89
20-23.9	4	1.81	9	1.84	13	1.83
24-27.9	3	1.86	17	1.83	20	1.83
28-31.9	2	1.30	8	1.90	10	1.78
32-35.9			8	1.80	8	1.80
36-39.9			6	1.81	6	1.81
40-43.9			1	2.01	1	2.01
44-47.9			2	1.94	2	1.94
48-51.9			1	2.06	1	2.06

[a] Combined CSFP for deficient and control rats.

TABLE 6. Incidence of squamous metaplasia of the nasolacrimal duct according to plasma vitamin A concentration intervals.

Plasma vitamin A interval	Incidence of squamous metaplasia					
	Deficient		Control		Combined[a]	
	Number of rats	Percent positive	Number of rats	Percent positive	Number of rats	Percent positive
(μg/100 ml)		(%)		(%)		(%)
0-3.9	19	94.7 (76.7)[b]			19	94.7 (76.7)[b]
4-7.9	10	40.0 (39.2)			10	40.0 (39.2)
8-11.9	5	20.0 (26.6)			5	20.0 (26.6)
12-15.9	2	0.0 (0.0)	1	0.0 (0.0)[b]	3	0.0 (0.0)
16-19.9	9	11.1 (19.5)	2	0.0 (0.0)	11	9.1 (17.6)
20-23.9	4	25.0 (30.0)	10	0.0 (0.0)	14	7.1 (15.4)
24-27.9	3	0.0 (0.0)	17	11.8 (20.1)	20	10.0 (18.4)
28-31.9	2	0.0 (0.0)	8	12.5 (20.7)	10	10.0 (18.4)
32-35.9			8	25.0 (30.0)	8	25.0 (30.0)
36-39.9			6	16.7 (24.1)	6	16.7 (24.1)
40-43.9			1	0.0 (0.0)	1	0.0 (0.0)
44-47.9			2	50.0 (45.0)	2	50.0 (45.0)
48-51.9			1	0.0 (0.0)	1	0.0 (0.0)

[a] Combined incidence for deficient and control rats.

[b] Arcsin $\sqrt{\text{percent positive}}$.

TABLE 7. Cerebrospinal fluid pressure according to $\log_{10}$ liver vitamin A concentration intervals.

$\log_{10}$ liver vitamin A interval	Cerebrospinal fluid pressure					
	Deficient		Control		Combined[a]	
	Number of rats	Average	Number of rats	Average	Number of rats	Average
($\mu g/g\ 10^2$)		($\log_{10}$ mm saline)		($\log_{10}$ mm saline)		($\log_{10}$ mm saline)
0.0-0.29	7	2.28			7	2.28
0.3-0.59	9	2.15			9	2.15
0.6-0.89	10	2.17			10	2.17
0.9-1.19	4	1.98			4	1.98
1.2-1.49	3	1.83	2	1.82	5	1.83
1.5-1.79	1	2.02	7	1.92	8	1.93
1.8-2.09	4	1.98	5	1.84	9	1.90
2.1-2.39	5	1.81	5	1.97	10	1.89
2.4-2.69	2	1.54	9	1.80	11	1.75
2.7-2.99	7	1.76	17	1.80	24	1.78
3.0-3.29	2	1.26	10	1.87	12	1.77

[a] Combined for deficient and control rats.

TABLE 8. Incidence of squamous metaplasia of the nasolacrimal duct according to $\log_{10}$ liver vitamin A concentration intervals.

$\log_{10}$ liver vitamin A interval	Incidence of squamous metaplasia					
	Deficient		Control		Combined[a]	
	Number of rats	Percent positive	Number of rats	Percent positive	Number of rats	Percent positive
($\mu g/g\ 10^2$)		(%)		(%)		(%)
0.0-0.29	7	85.7 (65.8)[b]			7	85.7 (67.8)[b]
0.3-0.59	9	100.0 (90.0)			9	100.0 (90.0)
0.6-0.89	10	70.0 (56.8)			10	70.0 (56.8)
0.9-1.19	4	25.0 (30.0)			4	25.0 (30.0)
1.2-1.49	3	0.0 (0.0)	2	0.0 (0.0)[b]	5	0.0 (0.0)
1.5-1.79	1	0.0 (0.0)	7	0.0 (0.0)	8	0.0 (0.0)
1.8-2.09	4	0.0 (0.0)	5	0.0 (0.0)	9	0.0 (0.0)
2.1-2.39	5	0.0 (0.0)	5	0.0 (0.0)	10	0.0 (0.0)
2.4-2.69	2	50.0 (45.0)	9	11.1 (19.5)	11	18.2 (25.2)
2.7-2.99	7	14.3 (22.2)	17	23.5 (29.0)	24	20.8 (27.1)
3.0-3.29	2	0.0 (0.0)	11	18.2 (25.2)	13	15.4 (23.1)

[a] Combined incidence deficient and control rats.

[b] Arcsin $\sqrt{\text{percent positive}}$

Experiment II - chronic hypovitaminosis A. Table 9 contains the feed consumption and growth of the rats fed ad libitum. Feed consumption in g/day, Y_1, adjusted for body weight at the start of the comparison period, was found to be related to the $\log_{10}$ of vitamin A intake in µg/kg body weight/day, X, as follows:

$$Y_1 = 19.4 + 3.481(X-1.36)\theta \pm 1.2$$

where $\theta = 1$ when ≤ 1.36 and $\theta = 0$ when $X > 1.36$.

Terminal body weight in g, Y_2 and body weight gain adjusted for body weight at the start of the comparison period in g/day, Y_3, as functions of vitamin A intake were respectively:

$$Y_2 = 355 + 81.7669(X-1.34)\theta \pm 21$$

where $\theta = 1$ when $X \leq 1.34$ and $\theta = 0$ when $X > 1.34$

and $$Y_3 = 3.9 + 1.9993\ (X-1.35)\theta \pm 0.5$$

where $\theta = 1$ when $X \leq 1.35$ and $\theta = 0$ when $X > 1.35$. At vitamin A intakes exceeding 23 µg for feed consumed, 22 for terminal body weight and 23 for gain, these response criteria were unaffected by intake of the vitamin A, but at intakes less than these values, the responses decreased.

Plasma vitamin A concentrations in µg/100 ml, Y_4, and liver vitamin A concentrations in $\log_{10}$ µg/g 10^2, Y_5, presented in Table 10, were related to vitamin A intake as follows:

$$Y_4 = 1.3-3.144X+7.830X^2$$

and $$Y_5 = 1.83-2.8968X+1.498X^2 \pm 0.28$$

The responses of these two variables were minimal from the 4 µg to 16 µg intake, but from 16 µg to 256 µg the responses increased at essentially a linear rate. Plasma vitamin A concentrations according to $\log_{10}$ liver vitamin A concentration intervals, Table 11, tended to be linear.

Cerebrospinal fluid pressure expressed as $\log_{10}$ (millimeters of saline),

Y_6, and given in Table 10 was essentially equal at vitamin A intakes of 64 and 256 µg but greater at intakes of 4 and 16 µg. The relationship of this variable with $\log_{10}$ of vitamin A intake was:

$$Y_6 = 1.92-0.6899\ (X-1.39)\Theta \pm 0.14$$

where $\Theta = 1$ when $X \leq 1.39$ and $\Theta = 0$ when $X > 1.39$.

At vitamin A intakes greater than 25 µg, the pressure was maintained at a geometric mean equal to 83 mm. For each 10% decrease in vitamin A intake below 25 µg, cerebrospinal fluid pressure increased at a rate of 8%. Squamous metaplasia of the nasolacrimal duct, Table 10, occurred in all four of the rats fed either the 4 or 16 µg intake and in one rat fed the highest intake of 256 µg.

Average cerebrospinal fluid pressure and incidence of squamous metaplasia of the nasolacrimal duct according to successive intervals of plasma vitamin A and of $\log_{10}$ liver vitamin A concentrations are given respectively in Tables 12 and 13. Since there were too few rats per interval, no attempt was made to determine the possible association between these variables as was done in experiment I.

TABLE 9. Effects of graded intakes of vitamin A upon feed consumption and body weight gain in the weanling male rat.

Criteria	Vitamin A intake - μg/kg body weight/day[a]				SD per rat
	4	16	64	256	
Animals, no	4	4	4	4	---
Feed, g/d					
Offered	23.0	22.8	23.3	22.3	1.4
Consumed					
Actual	17.2	18.7	19.5	19.0	1.2
Adjusted[b]	16.8	18.8	19.5	19.3	1.2
Body weight					
Initial, g	204	188	192	182	17
Terminal, g	295	344	355	356	21
Gain, g/d					
Actual	2.1	3.7	3.8	4.1	0.6
Adjusted[b]	2.3	3.6	3.8	4.0	0.5

[a] Expressed as retinol but fed as retinyl acetate.

[b] Adjusted for initial body weight at the start of the comparison period.

TABLE 10. Effects of graded intakes of vitamin A upon plasma and liver vitamin A concentration, cerebrospinal fluid pressure and incidence of squamous metaplasia, in the weanling male rat.

Criteria	Vitamin A intake - µg/kg body weight/day.[a] 4	16	64	256	SD per rat
Animals, no	4	4	4	4	---
Terminal plasma vitamin A, µg/100 ml	3 (2-4)[b]	7 (7-8)	23 (19-26)	39 (33-43)	---
Liver vitamin A, µg/g					
Actual	0.049	0.170	0.357	35.093	---
Log_{10}[c]	0.62	0.58	1.42	3.54	0.28
Cerebrospinal fluid pressure, mm saline					
Actual	306	116	86	88[d]	---
Log_{10}	2.46	2.05	1.92	1.92	0.14
Incidence of squamous metaplasia					
Percent	100	100	0	25	
Arcsin $\sqrt{\%\ positive}$	90	90	0	30	

[a] See Table 9.

[b] Range

[c] Actual liver vitamin A times 10^2

[d] 3 rats instead of 4.

TABLE 11. Plasma vitamin A concentration according to $\log_{10}$ liver vitamin A concentration interval.

$\log_{10}$ liver vitamin A interval	Number of rats	Plasma vitamin A
($\mu g/g\ 10^2$)		($\mu g/100$ ml)
0.3-0.59	4	5 (3-8)[a]
0.6-0.89	3	6 (4-8)
0.9-1.19	2	10 (2-19)
1.2-1.49	2	23 (20-25)
1.8-2.09	1	26
3.3-3.59	3	38 (33-43)
3.6-3.89	1	40

[a] Range.

TABLE 12. Cerebrospinal fluid pressure and incidence of squamous metaplasia of the nasolacrimal duct according to plasma vitamin A concentration intervals.

Plasma vitamin A interval	Cerebrospinal fluid pressure		Incidence of squamous metaplasia	
	Number of rats	Average	Number of rats	Percent positive
(ug/100 ml)		($\log_{10}$ mm of saline)		(%)
0-3.9	4	2.46	4	100(90.0)[a]
4-7.9	3	1.99	3	100(90.0)
8-11.9	1	2.22	1	100(90.0)
16-19.9	1	2.06	1	0(0.0)
20-23.9	1	1.86	1	0(0.0)
24-27.9	2	1.88	2	0(0.0)
32-35.9	1	2.13	1	0(0.0)
36-39.9	1	1.79	1	0(0.0)
40-43.9	1	1.83	2	50(45.0)

[a] Arcsin $\sqrt{\text{percent positive}}$

TABLE 13. Cerebrospinal fluid pressure and incidence of squamous metaplasia of the nasolacrimal duct according to $\log_{10}$ liver vitamin A concentration intervals.

$\log_{10}$ liver vitamin A interval	Cerebrospinal fluid pressure		Incidence of squamous metaplasia	
	Number of rats	Average	Number of rats	Percent positive
($\mu g/g\ 10^2$)		($\log_{10}$ mm saline)		(%)
0.3-0.59	4	2.24	4	100(90.0)[a]
0.6-0.89	3	2.15	3	100(90.0)
0.9-1.19	2	2.36	2	50(45.0)
1.2-1.49	2	1.88	2	0(0.0)
1.8 -2.09	1	1.86	1	0(0.0)
3.3-3.59	2	1.96	3	33(35.1)
3.6-3.89	1	1.83	1	0(0.0)

[a] Arcsin $\sqrt{\text{percent positive}}$

Experiment III - chronic hypovitaminosis A. Feed consumption and growth statistics of the rats which were equalized fed are contained in Table 14. Feed consumption in g/day, Y_1, adjusted for body weight at the start of the comparison period was related to $\log_{10}$ vitamin A intake in µg/kg body weight/day as follows:

$$Y_1 = 11.5 + 1.0834(X-1.41)\theta \pm 0.6$$

in which $\theta = 1$ when $X \leq 1.41$ and $\theta = 0$ when $X > 1.41$. Similar functions for terminal body weight in g, Y_2, and body weight gain in g/day, Y_3, both adjusted for body weight at the start of the comparison period were, respectively:

$$Y_2 = 194 + 44.7090(X-1.44)\theta \pm 22$$

where $\theta = 1$ when $X \leq 1.44$ and $\theta = 0$ when $X > 1.44$

and $$Y_3 = 1.5 + 1.2999(X-1.43)\theta \pm 0.6$$

where $\theta = 1$ when $X \leq 1.43$ and $\theta = 0$ when $X > 1.43$. Above intakes for Y_1 of 26 µg for Y_2 of 28 µg and for Y_3 of 27 µg, vitamin A intake had no significant effect. Below these intakes, all three criteria decreased linearly with decreasing intakes of the vitamin.

The responses, Table 15, of plasma vitamin A in µg/100 ml, Y_4, and of $\log_{10}$ liver vitamin A in µg/g 10^2, Y_5, were

$$Y_4 = 2.4-4.246X + 5.006X^2$$

and $$Y_5 = 2.03-3.3357X + 1.6136X^2 \pm 0.35.$$

The responses were inappreciable as the intake of vitamin A was increased up to 12 µg intake level. Thereafter the responses increased at increasing rates with increases in $\log_{10}$ vitamin A intake. Plasma vitamin A concentrations according to $\log_{10}$ vitamin A intervals, Table 16, were curvilinear, exhibiting little increase at the lower $\log_{10}$ liver vitamin A concentration intervals and tending to plateau and then decrease at the higher intervals.

Cerebrospinal fluid pressure, Y_6, expressed as $\log_{10}$ mm of saline was higher at intakes of vitamin A of 4, 12 and 16 µg than at 64 and 256 µg, Table 15. The geometric mean pressure of the three intakes averaged 79 mm of saline and of the 64 and 256 µg intakes 55 mm of saline. The incidence of squamous metaplasia of the nasolacrimal duct, Y_7, expressed as arcsin $\sqrt{\%}$, was found to be related to vitamin A intake, X, as follows:

$$Y_7 = 9.8 - 93.3993(X-1.51)\Theta \pm 63.8$$

in which $\Theta = 1$ when $X \leq 1.51$ and $\Theta = 0$ when $X > 1.51$. At intakes above 32 µg the incidence was estimated to be unaffected and averaged 3%. At intakes less than 32 µg, the incidence increased with decreasing intake, being estimated to be 38% at the 16 µg intake and 59% at the 8 µg intake.

Cerebrospinal fluid pressure averages and incidence of squamous metaplasia of the nasolacrimal duct, expressed respectively as $\log_{10}$ mm of saline, Y_6, and arcsin $\sqrt{\text{percent positive}}$, Y_7, according to plasma vitamin A concentration intervals, Y_4, and according to $\log_{10}$, liver vitamin A concentration intervals, Y_5, are given in Tables 16 and 17. The broken-line regression of the relationship between cerebrospinal fluid pressure and plasma vitamin A was not significant, but the relationship for incidence of squamous metaplasia was and is described as follows:

$$Y_7 = 0.0-6.3385(Y_4-12.4)\Theta \pm 23.93, \quad R^2 = 0.97$$

in which $\Theta = 1$ when $Y_4 \leq 12.4$ and $\Theta = 0$ when $Y_4 > 12.4$. At plasma vitamin A concentrations exceeding 12.4, the incidence of squamous metaplasia was zero. When the concentrations were less than 12.4, the incidence increased with decreasing concentration and was estimated to equal 7, 42 and 84% respectively at plasma concentrations of 10, 6 and 2 µg. The broken-line regression of the relationship between cerebrospinal fluid pressure and $\log_{10}$ liver vitamin A concentration was not significant, but the equation for incidence of squamous metaplasia was significant and is as follows:

$$Y_7 = 0.0-22.5532(Y_5 - 2.66)\Theta \pm 15.2,\ R^2 = 0.97$$

in which $\Theta = 1$ when $Y_5 \leq 2.66$ and $\Theta = 0$ when $Y_5 > 2.66$. Above liver vitamin A concentrations of 4.6 µg/g (antilog of 2.66 times 10^{-2}), there was no squamous metaplasia present in the rats. Below this liver vitamin A concentration, the incidence increased and was estimated to be 7, 13 and 37% respectively at liver vitamin A concentrations equivalent to 1, 0.5 and 0.1 µg/g.

TABLE 14. Effects of graded levels of vitamin A upon feed consumption and body weight gain in the weanling male rat.

Criteria	Vitamin A intake, μg/kg body weight/day[a]					SD per rat
	4	12	16	64	256	
Animals, no	6	9	8	9	9	
Feed, g/d						
Offered	11.2	11.2	11.4	11.7	11.5	1.7
Consumed						
Actual	10.6	11.1	11.4	11.7	11.5	1.7
Adjusted[b]	10.7	11.1	11.4	11.6	11.5	0.7
Body weight						
Initial, g	142	142	141	142	141	18
Terminal, g						
Actual	157	176	185	194	194	36
Adjusted[b]	157	176	185	193	195	22
Gain, g/d						
Actual	0.4	1.0	1.3	1.5	1.5	0.7
Adjusted[b]	0.4	1.0	1.3	1.5	1.5	0.6

[a] Expressed as retinol but fed as retinyl acetate.

[b] Adjusted for initial body weight at the start of the comparison period.

TABLE 15. Effects of graded levels of vitamin A upon plasma and liver vitamin A, cerebrospinal fluid pressure and incidence of squamous metaplasia in the weanling male rat.

Criteria	Vitamin A intake, μg/kg body weight/day[a]					SD per rat
	4	12	16	64	256	
Animals, no	6	9	8	9	9	---
Terminal plasma vitamin A, μg/100 ml	2 (1-4)[c]	3 (2-4)	4 (2-8)	12[b] (10-18)	21 (14-27)	---
Liver vitamin A, μg/g						
Actual	0.05[e]	0.03	0.04	0.14	28.48	---
Log_{10}[d]	0.52	0.37	0.50	1.07	3.42	0.35
Cerebrospinal fluid pressure, mm saline[f]						
Actual	80	55	94	66	56	---
Log_{10}	1.89	1.90	1.90	1.78	1.70	0.20
Incidence of squamous[g] metaplasia						
Actual	100.0	88.9	12.5	11.1	0.0	---
Arcsin $\sqrt{\text{percent}}$	90.0	70.5	20.7	19.5	0.0	---

[a] See Table 14.

[b] 8 rats instead of 9.

[c] Range.

[d] Actual liver vitamin A times 10^2.

[e] 8 rats instead of 6.

[f] 5 rats instead of 6 for 4 μg level.
7 rats instead of 8 for 16 μg level.
8 rats instead of 9 for 256 μg level.

[g] 8 rats instead of 6 for 4 μg level.

TABLE 16. Plasma vitamin A concentration according to $\log_{10}$ liver vitamin A concentration interval.

$\log_{10}$ liver vitamin A interval	Number of rats	Plasma vitamin A
($\mu g/g\ 10^2$)		($\mu g/100$ ml)
0.0-0.29	9	3 (1-5)[a]
0.3-0.59	6	3 (2-4)
0.6-0.89	7	6 (1-12)
0.9-1.19	5	6 (4-10)
1.2-1.49	4	13 (10-18)
3.0-3.29	2	24 (21-27)
3.3-3.59	4	20 (14-26)
3.6-3.89	3	20 (15-24)

[a] Range

TABLE 17. Cerebrospinal fluid pressure and incidence of squamous metaplasia of the nasolacrimal duct according to plasma vitamin A concentration intervals.

Plasma vitamin A interval	Cerebrospinal fluid pressure		Incidence of squamous metaplasia	
	Number of rats	Average	Number of rats	Percent positive
($\mu g/100$ ml)		($\log_{10}$ mm of saline)		(%)
0 - 3.9	15	1.90	16	87.5(69.3)[a]
4 - 7.9	5	1.83	6	16.6(24.0)
8 - 11.9	6	1.88	6	16.6(24.0)
12 - 15.9	4	1.70	4	0.0(0.0)
16 - 19.9	2	1.66	2	0.0(0.0)
20 - 23.9	3	1.85	4	0.0(0.0)
24 - 27.9	2	1.52	2	0.0(0.0)

[a] Arcsin $\sqrt{\text{percent positive}}$

TABLE 18. Cerebrospinal fluid pressure and incidence of squamous metaplasia of the nasolacrimal duct according to $\log_{10}$ liver vitamin A concentration intervals.

	Cerebrospinal fluid pressure		Incidence of squamous metaplasia	
$\log_{10}$ liver vitamin A interval	Number of rats	Average	Number of rats	Percent positive
($\mu g/g\ 10^2$)		($\log_{10}$ mm of saline)		(%)
0.0–0.29	8	1.82	9	66.6(54.7)[a]
0.3–0.59	6	1.96	7	71.4(57.7)
0.6–0.89	7	1.76	7	42.8(40.9)
0.9–1.19	4	1.95	5	20.0(26.6)
1.2–1.49	5	1.89	6	33.3(35.2)
3.0–3.29	2	1.72	2	0.0(0.0)
3.3–3.59	4	1.62	4	0.0(0.0)
3.6–3.89	2	1.82	2	0.0(0.0)

[a] Arcsin $\sqrt{\text{percent positive}}$

Experiment IV - chronic hypovitaminosis A. Feed consumption and growth of rats equalized-fed, but at a higher intake than those of experiment III, are contained in Table 19. Adjusted feed consumption in g/day, Y_1, as a function of vitamin A intake in µg/kg body weight/day, X, was:

$$Y_1 = 14.4 + 2.0627\ (X-1.65)\theta \pm 1.0$$

where $\theta = 1$ when $X \leq 1.65$ and $\theta = 0$ when $X > 1.65$. Similar broken-line regressions for adjusted terminal body weight in g, Y_2, and adjusted body weight gain in g/day, Y_3 were respectively:

$$Y_2 = 254 + 74.9201(X-1.54)\theta \pm 23$$

where $\theta = 1$ when $X \leq 1.54$ and $\theta = 0$ when $X > 1.54$

and $$Y_3 = 2.8 + 2.2769\ (X-1.51)\theta \pm 0.7$$

where $\theta = 1$ when $X < 1.51$ and $\theta = 0$ when $X > 1.51$. At vitamin A intakes exceeding 45 µg for feed consumption, 35 µg for terminal body weight and 32 µg for body weight gain, intake of the vitamin had inappreciable effect. In contrast when lesser intakes were fed, all three criterion decreased linearly.

Plasma vitamin A in µg/100 ml, Y_4, and liver vitamin A expressed as $\log_{10}$ µg/g 10^2, Y_5, increased slightly or was unaffected by increases in vitamin A intakes from 4 to 20 µg, but increased markedly at intakes from 20 to 256 µg, Table 20. Quadratic regressions of the responses of the two variables with increasing vitamin A intake were:

$$Y_4 = 1.4-4.227X + 5.174X^2$$

and $$Y_5 = 3.42 -4.905\ X + 2.034X^2 \pm 0.31$$

As in the previous experiments, the magnitude of the variation in plasma vitamin A concentration increased as vitamin A intake increased. Plasma vitamin A concentration according to $\log_{10}$ liver vitamin A concentrations appeared to be linear, Table 21.

Cerebrospinal fluid pressure, Table 20, expressed as $\log_{10}$ mm of saline, Y_6, was related to vitamin A intake in $\log_{10}$ µg/kg body weight/day, X, as follows:

$$Y_6 = 1.73-0.3698(X-1.68)\theta \pm 0.15$$

where $\theta = 1$ when $X \leq 1.68$ and $\theta = 0$ when $X > 1.68$. Above intakes of vitamin A of 48 µg, the pressure was maintained at a geometric mean of 54 mm. Below the 48 µg intake, each 10% decrease in intake resulted in a 1.9% increase in pressure.

Squamous metaplasia of the nasolacrimal duct, Table 20, expressed as arcsin $\sqrt{\text{percent positive}}$, Y_7, was associated with vitamin A intake, X, not including the 8 µg intake group data, as follows:

$$Y_7 = 0.0-280.96(X-1.40)\theta \pm 1.2$$

where $\theta = 1$ when $X \leq 1.40$ and $\theta = 0$ when $X > 1.40$. At vitamin A intakes exceeding 25 µg, the incidence was 0. At vitamin A intakes of 20 and 15 µg, the incidence was estimated to be 31.8 and 52.5%, respectively. Since the broken-line regression equation can only delineate one area of linear change and one area of no change of a variable in relation to another variable and both the 8 and 12 µg intakes had identical % positive values, 100, the 8 µg intake % was not utilized in the derivation of the equation.

The 2nd lumbar vertebrate total volume, Y_8, canal volume, Y_9, both in cm^3, and the ratio of canal volume to total volume times 10^2 are reported in Table 20. The rats fed the vitamin A intakes of 8-20 µg had smaller canal volumes and ratios of canal volume to total volume than the rats fed the 64 and 256 µg intakes. Upon plotting the data, the magnitude of total and canal volumes within a vitamin A intake group were related to body weight at the end of the comparison period but the ratios were independent of body weight. The means for total volume adjusted for body weight and in order of increasing vitamin A intakes were, respectively, for total volume, 0.109, 0.108, 0.109, 0.102,

0.102 and 0.091 cm^3 with a SD = 0.012 and for canal volume, 0.047, 0.042, 0.046, 0.045, 0.047 and 0.047 cm^3 with a SD = 0.007.

Average cerebrospinal fluid pressure expressed as $\log_{10}$ mm of saline, Y_6, and incidence of squamous metaplasia of the nasolacrimal duct, expressed as arcsin $\sqrt{\text{percent positive}}$, Y_7, according to plasma vitamin A, Y_4, concentration intervals and according to $\log_{10}$ liver vitamin A, Y_5, concentration intervals are given, respectively, in Tables 22 and 23. Cerebrospinal fluid pressure and incidence of squamous metaplasia were related to plasma vitamin A concentration as follows:

$$Y_6 = 1.72 - 0.0282(Y_4-10.7)\Theta \pm 0.10, \quad R^2 = 0.97$$

where $\Theta = 1$ when $X \leq 10.7$ and $\Theta = 0$ when $Y_4 > 10.7$ and

$$Y_7 = 0.0-10.7064(Y_4-8.7)\Theta \pm 0.0, \quad R^2 = 1.00$$

where $\Theta = 1$ when $Y_4 \leq 8.7$ and $\Theta = 0$ when $Y_4 > 8.7$. The pressures were maintained at a geometric mean of 52 mm of saline at plasma vitamin A concentrations greater than 10.7 µg. For each 10% decrease in concentration of plasma vitamin A below 10.7 µg, the cerebrospinal fluid pressure increased 7%. The incidence of squamous metaplasia was zero at plasma vitamin A concentrations exceeding 8.7 µg and increased at concentrations less than 8.7 µg, being predicted to be equivalent to 23 and 59% respectively at plasma concentrations of 6 and 4 µg. Neither cerebrospinal fluid pressure nor incidence of squamous metaplasia of the nasolacrimal duct was found to be significantly related to liver vitamin A concentration when the broken-line regression model was utilized.

TABLE 19. Effects of graded intakes of vitamin A upon feed consumption and body weight gain in the weanling male rat.

Criteria	Vitamin A intake, μg/kg body weight/day[a]						SD per rat
	8	12	16	20	64	256	
Animals, no	7	8	9	9	9	9	---
Feed, g/d							
Offered	11.8	14.3	13.5	13.7	16.0	14.8	2.1
Consumed							
Actual	11.2	13.8	13.2	13.4	15.7	14.7	2.0
Adjusted[b]	12.6	13.6	13.5	13.5	14.4	14.4	1.0
Body weight							
Initial, g	140	159	154	155	170	159	21
Terminal, g							
Actual	175	232	224	234	278	255	40
Adjusted[b]	201	228	227	234	256	252	23
Gain, g/d							
Actual	1.0	2.1	2.0	2.2	3.1	2.8	0.7
Adjusted[b]	1.3	2.0	2.0	2.3	2.9	2.7	0.7

[a] Expressed as retinol but fed as retinyl acetate.

[b] Adjusted for initial body weight at the start of the comparison period.

TABLE 20. Effects of graded intakes of vitamin A upon plasma and liver vitamin A concentration, cerebrospinal fluid pressure, incidence of squamous metaplasia and the 2nd lumbar vertebrate total and canal volume.

Criteria	Vitamin A intake, µg/kg body weight/day[a]						SD per rat
	8	12	16	20	64	256	
Animals, no	7	8	9	9	9	9	---
Terminal plasma vitamin A, µg/100 ml	2 (2-4)[b]	3 (2-4)	4 (2-7)	7 (4-14)	12 (10-16)	24 (22-27)	---
Liver vitamin A, µg/g							
Actual	0.08	0.02	0.03	0.07	0.19	25.36	---
Log_{10}[c]	0.80	0.25	0.40	0.65	1.20	3.40	0.31
Cerebrospinal fluid pressure, mm saline							
Actual	106	108	88	69	53	60	---
Log_{10}	1.98	2.02	1.92	1.83	1.71	1.75	0.15
Incidence of squamous metaplasia							
Percent	100	100	67	22	0	0	---
Arcsin $\sqrt{\%}$ positive	90	90	55	28	0	0	---
Second lumbar vertebrate, cm^3							
Total volume	0.089	0.107	0.106	0.102	0.118	0.099	0.018
Canal volume	39	0.042	0.045	0.045	0.053	0.050	0.009
Canal volume to total volume[d]		39	44	44	46	50	8

[a] See Table 19.

[b] Range.

[c] Actual liver vitamin A times 10^2.

[d] Canal volume to total volume times 10^2.

TABLE 21. Plasma vitamin A concentration according to $\log_{10}$ liver vitamin A concentration interval.

$\log_{10}$ liver vitamin A interval	Plasma vitamin A	
	Number of rats	Average
($\mu g/g\ 10^2$)		($\mu g/100$ ml)
0.0-0.29	12	4 (2-8)[a]
0.3-0.59	5	4 (2-7)
0.6-0.89	11	4 (2-10)
0.9-1.19	8	3 (4-16)
1.2-1.49	4	11 (2-16)
1.5-1.79	2	12 (12-13)
3.3-3.59	9	24 (22-27)

[a] Range

TABLE 22. Cerebrospinal fluid pressure and incidence of squamous metaplasia of the nasolacrimal duct according to plasma vitamin A concentration intervals.

Plasma vitamin A interval	Cerebrospinal fluid pressure		Incidence of squamous metaplasia	
	Number of rats	Average	Number of rats	Percent positive
($\mu g/100$ ml)		($\log_{10}$ mm of saline)		(%)
0-3.9	23	1.97	23	91.3(72.8)[a]
4-7.9	7	1.87	7	25.0(25.0)
8-11.9	6	1.74	6	0.0(0.0)
12-15.9	2	1.62	2	0.0(0.0)
16-19.9	2	1.70	2	0.0(0.0)
20-23.9	4	1.72	4	0.0(0.0)
24-27.9	5	1.78	5	0.0(0.0)

[a] Arcsin $\sqrt{\text{percent positive}}$

TABLE 23. Cerebrospinal fluid pressure and incidence of squamous metaplasia of the nasolacrimal duct according to $\log_{10}$ liver vitamin A concentration interval.

$\log_{10}$ liver vitamin A interval	Cerebrospinal fluid pressure		Incidence of squamous metaplasia	
	Number of rats	Average	Number of rats	Percent positive
($\mu g/g\ 10^2$)		($\log_{10}$ mm of saline)		(%)
0.0-0.29	12	1.88	12	75.0(60.0)[a]
0.3-0.59	5	2.01	5	100.0(90.0)
0.6-0.89	11	1.95	11	72.7(58.5)
0.9-1.19	7	1.82	8	0.0(0.0)
1.2-1.49	3	1.77	4	25.0(30.0)
1.5-1.79	2	1.62	2	0.0(0.0)
3.3-3.59	9	1.75	9	0.0(0.0)

[a] Arcsin $\sqrt{\text{percent positive}}$

Experiment V - chronic hypovitaminosis A. Feed consumption and growth of the rats equalized-fed, but on body weight increase calculated from experiment IV rather than on a contemporary weight increase, are contained in Table 24. Rats fed the deficient vitamin A intake, 12 µg, consumed less feed than rats fed the control intake, 256 µg. This resulted in the deficient rats weighing and gaining less per day than the control rats.

As was expected, terminal plasma and liver vitamin A concentrations were considerably less in the deficient rats than in the controls, Table 25. Cerebrospinal fluid pressure was greater in the deficient animals. The total volume of the 2nd lumbar vertebrate for both the 6 and 8 comparison weeks deficient groups averaged 0.138 cm^3 and both the 6 and 8 comparison weeks control groups 0.138 cm^3. In contrast, the canal volume was less in the deficient rats as was the ratio of the canal volume to total volume.

TABLE 24. Effects of chronic vitamin A deficiency upon feed consumption and body weight in the weanling male rat.

	Comparison Period, weeks				
	6		8		
	Vitamin A intake, g/kg body weight/day[a]				SD per rat
	12	256	12	256	
Animals, no	9	10	8	10	---
Feed, g/d					
Offered	18.3	18.0	18.3	18.1	0.7
Consumed					
Actual	16.4	17.1	16.2	17.1	1.2
Adjusted[b]	16.0	17.0	16.2	17.4	0.9
Body weight					
Initial, g	168	158	156	147	25
Terminal, g					
Actual	284	304	285	336	31
Adjusted[b]	274	303	285	344	22
Gain, g/d	2.8	3.5	2.3	3.4	0.5

[a] Expressed as retinol but fed as retinyl acetate.

[b] Adjusted for initial body weight at start of comparison period.

TABLE 25. Effects of chronic vitamin A deficiency upon plasma and liver vitamin A concentrations, cerebrospinal fluid pressure and 2nd lumbar vertebrate total and canal volume

	Comparison Period, weeks				
	6		8		
	Vitamin A intake, μg/kg body weight/day[a]				SD per rat
	12	256	12	256	
Animals, no	9	10	8	10	---
Terminal plasma vitamin A, μg/100 ml	5 (3-6)[b]	33 (26-44)	7 (4-9)	38 (29-50)	---
Liver vitamin A, μg/g					
Actual	0.15	35.50	0.10	52.91	---
Log_{10}[c]	0.99	3.50	0.86	3.69	0.25
Cerebrospinal fluid[d] pressure, mm saline					
Actual	114	69	107	75	---
Log_{10}	2.03	1.83	2.01	1.87	0.14
2nd lumbar vertebrate, cm^3					
Total volume	0.138	0.122	0.129	0.138	0.018
Canal volume	0.029	0.043	0.030	0.045	0.009
Canal/total volume[e]	21	35	23	33	8

[a] See Table 24

[b] Range

[c] Actual liver vitamin A times 10^2

[d] Cerebrospinal fluid pressure based on 8 rather than 9 rats (6 week, 12 μg.)
7 rather than 8 rats (8 week, 12 μg.)
9 rather than 10 rats (8 week, 256 μg.)

[e] Times 10^2

The result of the acute hypovitaminosis A study are in agreement with those of previous investigators (6, 24) in that decreases in body weight gain or actual losses in body weight occur during vitamin A depletion later than do physiological or histological alterations. It should be noted that in none of the groups of deficient rats did the rate of growth reach a plateau, Appendix figure I, during the five week depletion period. Yet the cerebrospinal fluid pressure at the 3rd depletion week was greater than that of the control rats and incidence of squamous metaplasia of the nasolacrimal duct was also greater at this time.

Under the experimental conditions employed in these studies, a three week partial vitamin A depletion period prior to studying chronic hypovitaminosis A appeared to be repeatable with respect to plasma vitamin A concentration, Table 26. As can be seen from the statistics in column three of this table, plasma vitamin A concentration was remarkably consistent from experiment to experiment. In contrast liver stores, geometric means, varied by a factor of 4.7. There was little difference among experiments in terms of plasma or liver vitamin A concentrations of the weanling rats upon arrival, Table 26-first column and the same was essentially so for the concentrations at the completion of the one week standardizing period, Table 26-2nd column.

Chronic hypovitaminosis A in the rat under conditions of equalized feeding (equalized ration offered) has been demonstrated in the present experiments. In addition losses in body weight did not occur at retinol equivalent intakes of >8 μg/kg body weight/day, Appendix Figures II-V. Since requirements for vitamin A are related to growth and since rapid growth is necessary for the appearance of deficiency changes (6), a 12 μg intake under conditions of these studies would appear to be the minimum intake to utilize in producing a deficiency since the 8 μg intake resulted in growth plateau during the fifth

week of experiment IV, Appendix figure IV. Utilizing body weight increases from previous experiments, as was employed in experiment V, rather than contempory body weight increases, as was employed in experiments III and IV, would appear to be preferable, but more definitive information than is supplied from the present studies is needed to provide an unequivocal answer.

Elevated cerebrospinal fluid pressure, based upon the number of days required for development in rats fed a vitamin A-free diet or the intake below which it occurs in rats fed graded intakes of the vitamin, Table 27, appeared to be a more sensitive indicator of hypovitaminosis A than did either feed consumption or growth. The incidence of squamous metaplasia of the nasolacrimal duct was more variable than the other three criteria. While the greater sensitivity of elevated cerebrospinal fluid pressure agreed with previous observations from several species as recently reviewed by Corey and Hayes (6), the differences among criteria were relatively small in magnitude, In the experiments described herein, ration consumption and growth were both reduced in comparison to those of rats fed adequate vitamin A. A possibility for future work would be to estimate the rate of gain from data of mildly deficient rats, such as those fed a 12 μg intake, instead of rats fed deficient as well as control intakes (as was done in experiment V). Such a procedure might overcome the differences in feed consumption. Other possibilities include paired feedings or paired weight-gain feeding. Lastly, pattern of food consumption is generally different between deficient and control rats with deficient rats consuming their food at slower rates (4), which from casual observation occurred in the present experiments. This difference in rate of food consumption, meal eaters characteristic of the controls and nibblers characteristic of the deficient, probably accounts in part for the differences in body composition (22). Differences in pattern of food consumption might be overcome by forced feeding or by allotting feed to the controls at a

rate over time consistent with the pattern of food consumption of the deficient animals (27). However, in rapidly induced vitamin A deficiency, forced feeding did not result in equal growth between deficient and control rats (18).

The estimated requirements for vitamin A based on the amount necessary to prevent elevated cerebrospinal fluid pressure, increased incidence of squamous metaplasia of the nasolacrimal duct, or decreased feed consumption and growth varied among the three chronic experiments, Table 27, with the greatest difference being between experiments II and III and experiment IV. With respect to cerebrospinal fluid pressure, the requirements varied from 16 to 48 µg/kg body weight/day which when considered in toto was considerably greater than that, 11.8 µg, reported by Corey and Hayes (6). The estimated requirements for the prevention of squamous metaplasia of the nasolacrimal duct, 25-32 µg, were also greater than those, 6-9 µg, reported by Irving and Richards (16). In contrast, the amounts estimated to be required for feed consumption and growth, 22-45 µg, were less than the NRC requirement of 60 µg for minimal growth (20). The variability in these estimates are typical of the vitamin A literature (20) and are possibly due to variation in experimental procedures and analyses of data. For example, in the present experiments partial vitamin A depletion was undertaken prior to comparison of vitamin A intakes. Thus, when the data from the present experiments are compared to those from experiments without such depletion one would expect the estimated requirements to be greater in the former since animals not partially depleted of their vitamin A stores could utilize them during the comparison. In the present experiments, responses to vitamin A intake were considered to be a function of the logarithm to the base 10 of vitamin A intake (2) which is known to provide greater estimated requirements than when responses are considered to be a function of actual vitamin A intake. Lastly, numerous other factors affect vitamin A requirements of growing

animals (9) such as age, inheritance, intensity of feeding and growth, ration constituents, ration additives or contaminants, source of vitamin A, environment including ambient temperature and exposure to infective bacteria.

The degree of association between elevated cerebrospinal fluid pressure or increased incidence of squamous metaplasia of the nasolacrimal duct and plasma vitamin A concentration, Table 28, was reasonably consistent among experiments, but similar estimates for $\log_{10}$ liver vitamin A were quite variable. The results with respect to plasma vitamin A are in good agreement with those estimated by Corey and Hayes (6) for the rat. Apparently the rat requires considerably less circulating vitamin A to prevent the occurence of deficiency alterations than some other species. In the pig (15), calf (8, 10), and weanling rabbit (7), the minimum concentration of plasma vitamin A reported to be necessary to prevent elevated cerebrospinal fluid pressure was 15-17 μg/100 ml. However, for other criteria such as the prevention of night blindness, ocular papilledema and squamous metaplasia of the parotid duct, the estimated values for the calf range from 8 to 20 μg/100 ml (8).

TABLE 26. Plasma and $\log_{10}$ liver vitamin A concentrations of weanling male rats on arrival, at the completion of one week of standardization and at the completion of three weeks of partial vitamin A depletion.

Experiment		Arrival	End of standardizing period	End of 3-week partial vitamin A depletion
		Plasma vitamin A, μg/100 ml		
I	Mean	----	21	6
	SD		8	2
II	Mean	----	--	6
	SD			3
III	Mean	16	24	8
	SD	4	8	3
IV	Mean	17	21	7
	SD	2	6	3
V	Mean	19	18	8
	SD	7	7	3
		$\log_{10}$ liver vitamin A, μg/g 10^2		
I	Mean	--	--	0.80 (0.06)[a]
	SD			0.36
II	Mean	--	--	--
	SD			
III	Mean	3.38 (23.99)[a]	2.92 (8.32)[a]	0.98 (0.10)
	SD	0.09	0.16	0.45
IV	Mean	3.37 (23.44)	2.95 (8.91)	0.51 (0.03)
	SD	0.08	0.16	0.60
V	Mean	3.40 (25.12)	3.07 (11.75)	1.15 (0.14)
	SD	0.08	0.32	0.26

[a] μg/g.

TABLE 27. Sensitivity of feed consumption, growth, elevated cerebrospinal fluid pressure and incidence of squamous metaplasia of the nasolacrimal duct to hypovitaminosis A.

Experiment	Decreased feed consumption	Decreased growth rate	Elevated cerebrospinal fluid pressure	Increased incidence of squamous metaplasia
	(g/day)	(g/day)	($\log_{10}$ mm of saline)	(arcsin $\sqrt{\%}$ positive)
I-acute	31 days	35 days	21 days	20 days
II-chronic	$\leq$23 µg	$\leq$22 µg	$\leq$25 µg	—[a]
III-chronic	$\leq$26 µg	$\leq$28 µg	>16<64 µg	$\leq$32
IV-chronic	$\leq$45 µg	$\leq$35 µg	$\leq$48 µg	$\leq$25 µg

[a] Too few rats per vitamin A intake grouping to derive a meaningful function of incidence on intake of the vitamin.

TABLE 28. Plasma and liver vitamin A concentrations below which either elevated cerebrospinal fluid pressure or increased incidence of squamous metaplasia of the nasolacrimal duct occurred.

Experiment	Elevated cerebrospinal fluid pressure	Increased incidence of squamous metaplasia
	($\log_{10}$ mm of saline)	(arcsin $\sqrt{\%}$ positive)
Plasma vitamin A concentration, µg/100 ml		
I-acute	< 7.7 µg	< 8.1 µg
III-chronic	NS[a]	<12.4
IV-chronic	<10.7	<. 8.7
III & IV-chronic[b]	<13.9 µg	< 8.0 µg
Liver vitamin A concentration, $\log_{10}$ µg/g 10^2		
I-acute	< 2.54 (3.5)[c]	< 1.34 (0.2)[c]
III-chronic	NS	< 2.66 (4.6)
IV-chronic	NS	NS
III & IV-chronic[b]	< 3.01 (10.2)	< 2.03 (1.1)

[a] Not statistically significant.

[b] Based upon broken-line regression utilizing combined data from experiments III and IV.

[c] µg/g

SUMMARY

An acute hypovitaminosis A study was conducted with weanling male rats to ascertain the degree of partial vitamin A depletion which had little effect on feed consumption and growth and where the incidence of elevated cerebrospinal fluid pressure and squamous metaplasia of the nasolacrimal duct was minimal. For this study, weanling rats were fed a vitamin A-free basal diet or the same diet plus vitamin A for 35 days. Decreased feed consumption, decreased rate of body weight gain, elevated cerebrospinal fluid pressure and increased incidence of squamous metaplasia was estimated to occur, respectively, on the 31st, 35th, 21st and 20th days. Therefore under these experimental conditions, it was concluded that a 3 week partial vitamin A depletion period would meet the objective stated above.

Four chronic hypovitaminosis A studies were conducted with weanling male rats to develop an equalized feeding (equalized ration offered) regime. In all studies, the partial vitamin A depletion was followed by a comparison period during which graded levels of vitamin A were fed with the vitamin A-free basal ration. In the initial experiment, the rats were fed ad libitum so as to provide feed consumption data for use in subsequent experiments. In the next two experiments, equalized feeding was based upon contemporary week to week body weight of the animals and in the last study, it was based on rates of gain in body weight from the previous experiment. The estimated requirement for vitamin A, to prevent elevated cerebrospinal fluid pressure, was consistently higher than those for feed consumed or body weight gain. The requirements estimated for the prevention of squamous metaplasia of the nasolacrimal duct were more variable. In spite of the greater sensitivity of elevated cerebrospinal fluid pressure, the estimated requirements were not too different from criterion to criterion, thus in all experiments, reduced feed consumption and growth were observed in the deficient weanling rats. While equalized feeding was clearly feasible, equalized food consumption, as well as rate or pattern of food consumption, presents problems not answered in the present studies.

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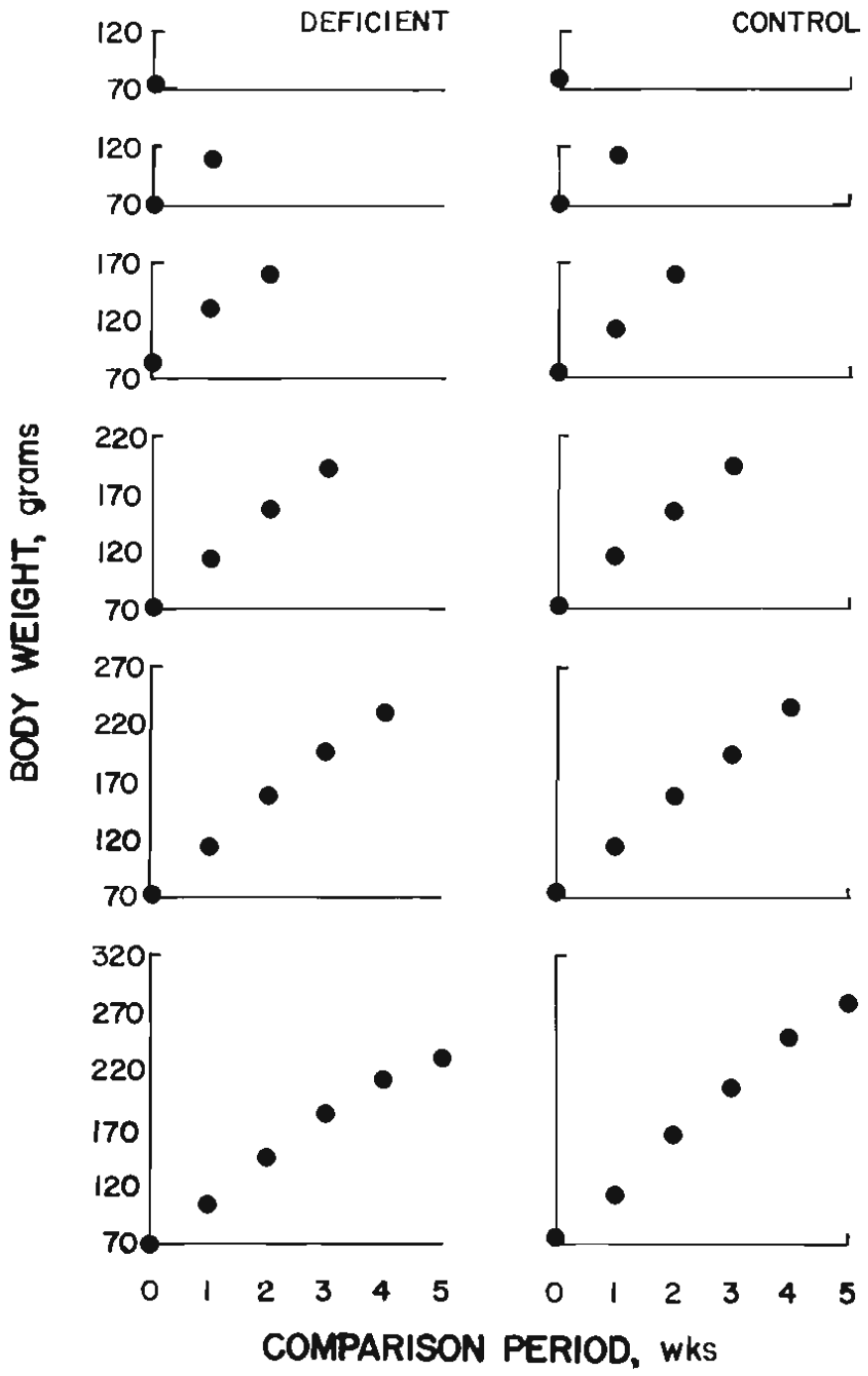
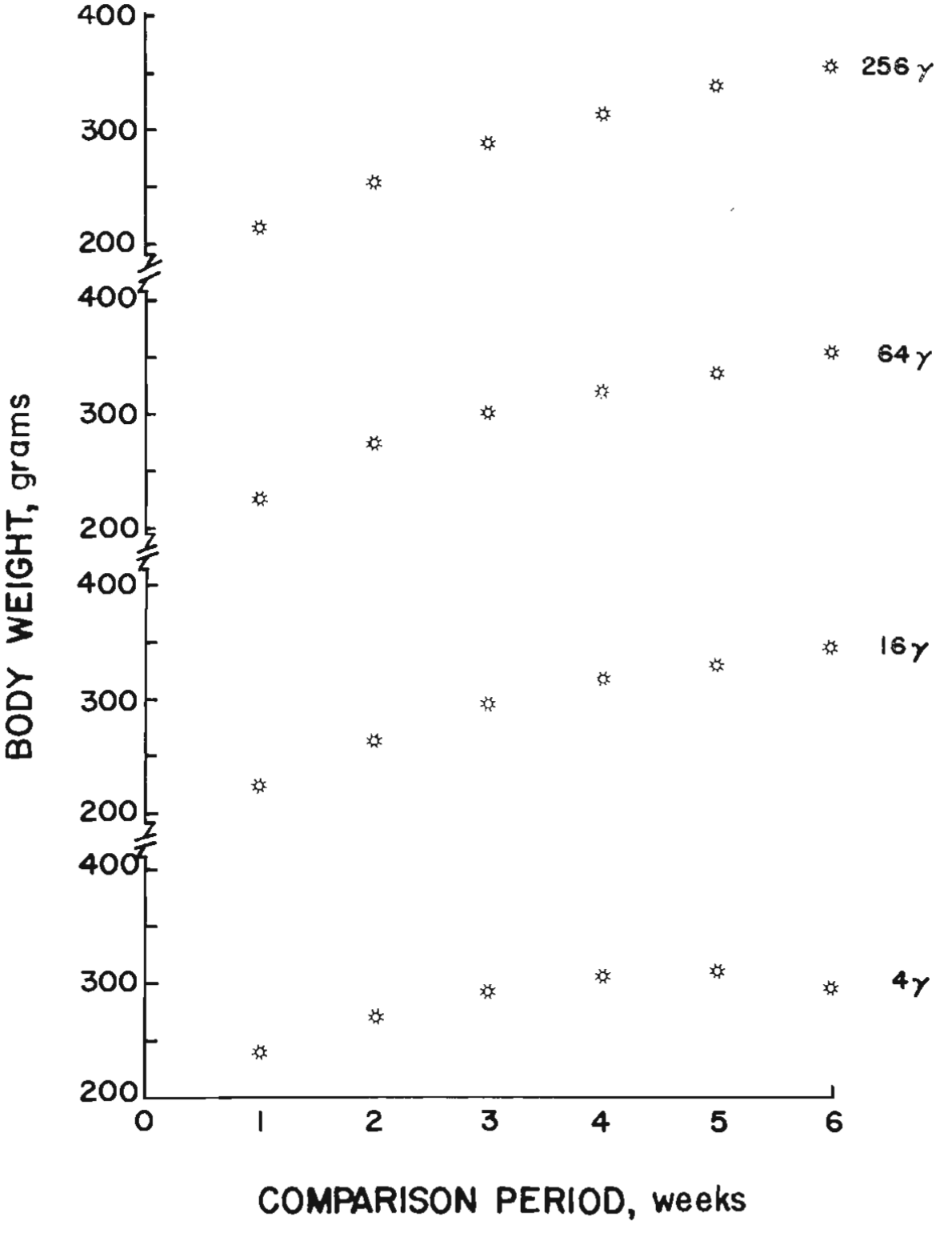
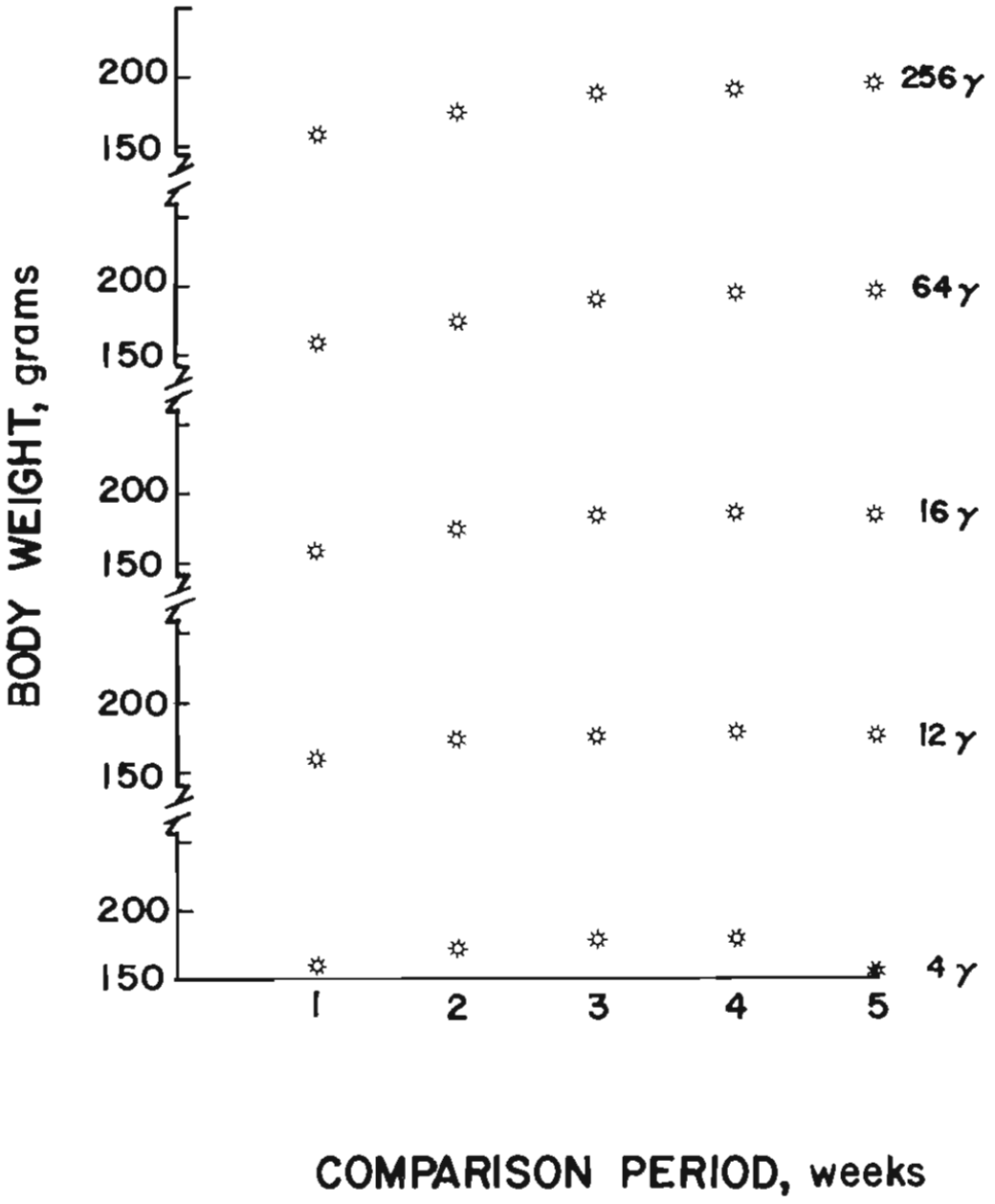


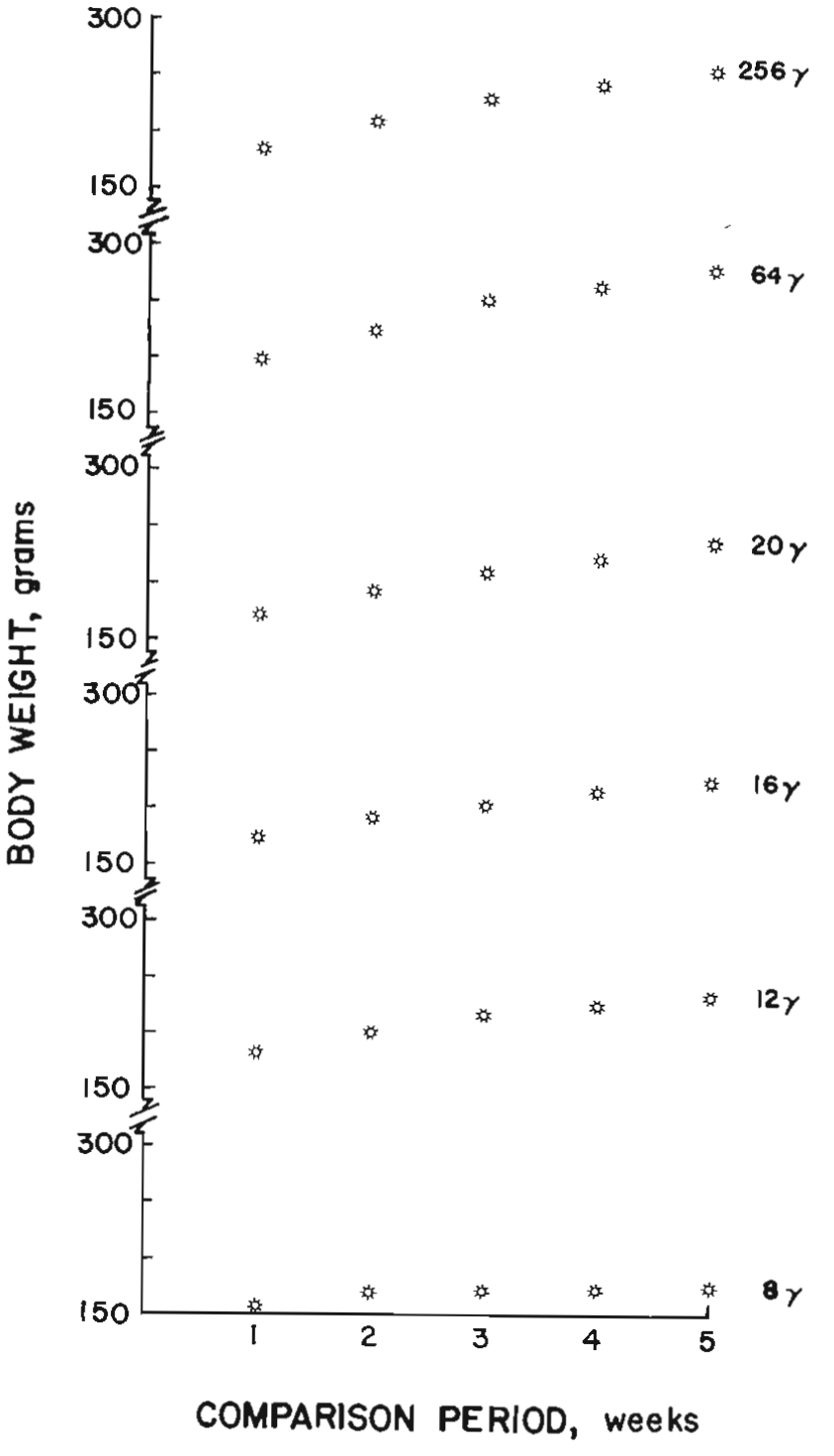
Appendix figure I. Experiment I - acute hypovitaminosis A. Rate of body weight gain during the comparison period for rats fed the basal ration without added vitamin A, deficient, or the basal ration containing 1 µg retinol/gram, control.



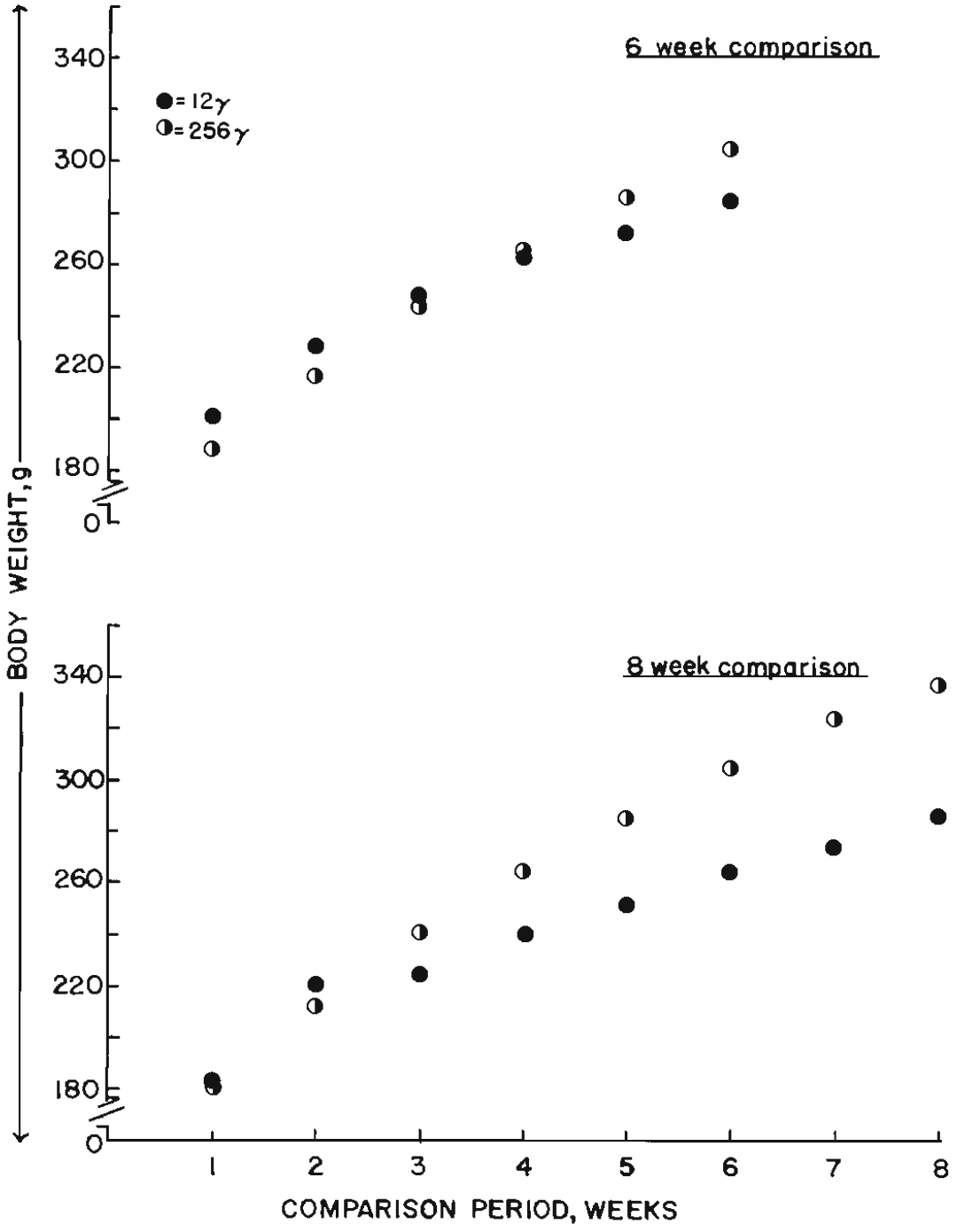
Appendix figure II. Experiment II - chronic hypovitaminosis A. Rate of body weight gain during the comparison period for rats fed ad libitum the basal ration plus graded levels of vitamin A.



Apprendix figure III. Experiment III - chronic hypovitaminosis A. Rate of body weight gain during the comparison period for rats equalized fed the basal ration plus graded levels of vitamin A.



Appendix figure IV. Experiment IV - chronic hypovitaminosis A. Rate of body weight gain during the comparison period for rats equalized fed the basal ration plus graded levels of vitamin A.



Appendix figure V. Experiment V - chronic hypovitaminosis. Comparison of body weight gain for rats equalized fed the basal ration plus 12 µg retinol/kg body weight/day, deficient, or 256 µg retinol/kg body weight/day, control, for a 6 or 8 week comparison period.