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RATE OF DEPLETION OF LIVER VITAMIN A IN CHICKS, WEANLING RABBITS AND WEANLING RATS FED VITAMIN A-FREE DIETS

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INTRODUCTION

The liver under "normal" dietary conditions has been demonstrated to be the major storage site for vitamin A in animals (1) and man (2). In addition, the liver has the ability to store considerable quantities of the vitamin which can provide the animal's needs for extended periods of time even though a diet deficient in vitamin A is fed (1). Saturation of the liver with vitamin A may be attained by massive toxic doses of the vitamin (3,4) but under usual dietary conditions it does not occur (1).

Interest in the rate of loss of liver stores of vitamin A has had several overall objectives. One of these was estimating losses in domestic animals fed rations containing low concentrations of carotenoids so as to estimate the time before it is necessary to add vitamin A or its precursor, carotene, to the ration (5-9). Another was for the purpose of estimating a single dosage of vitamin A to protect children from vitamin A deficiency during periods of inadequate vitamin A intake (10). In laboratory investigations it is often desired to partially deplete animals to a level of vitamin A storage which is just borderline in terms of deficiency so as to minimize the effects of the stored vitamin. Therefore estimates of rates of depletion of liver stores could be useful in ascertaining borderline deficiency status (11-13).

Various factors have been reported to influence the rate of loss of vitamin A and the early studies were adequately reviewed by Moore (1). Since Moore's review (1) researchers have continued to investigate vitamin A depletion rates and the effect upon the depletion rates of factors such as protein (14-17), environmental temperature (18), dietary retinoic acid (19), an oral contraceptive (20), dietary tocopherol (21), and dietary zinc (22).

The primary purpose of the present investigation was to estimate rates of loss of vitamin A from the liver of three species, the chick, the weanling male rabbit and the weanling male rat, currently being used in vitamin A research at this Station. Secondly, comparison between these estimates and those estimated from some previous studies reported in the literature were made.

METHODS

A. CHICKS

Experiment I. Sixty day-old White Rock male chicks were obtained from a local supplier (Arbor Acres, Glastonbury, CT.), wing-banded for identification purposes, randomly assigned to one of ten experimental lots with the restriction of six birds per lot and placed in ten pens of a battery (Petersime) located in the Processing Laboratory of the Roy E. Jones Building. Each lot was provided ad libitum a basal diet, Appendix Table I, to which was added retinyl acetate (Hoffmann-LaRoche Dry Vitamin A Acetate Beadlets, type 325-40 guaranteed to contain 111.8 mg retinyl acetate per gram) equivalent to 5 mg of retinol per kilogram of ration. After a two day standardization period on the above regime, one-half of the lots were assigned randomly to one of two dietary treatments; either the basal diet or the basal diet plus the 5 mg equivalent of retinol per kilogram of diet. At this time each lot of birds was reduced in size from six to five by elimination of the lightest bird from each lot. In addition, within the five lots fed the basal diet alone and within the five lots fed the basal diet plus vitamin A, one lot each was assigned to one of five time periods, 0, 1, 2, 3 or 4 weeks.

At the completion of each of the designated time periods, the chicks of each particular lot were sacrificed. One week after completion of this first replication of the experimental design, the procedure was repeated with an additional 60 chicks providing two complete replications of the experimental design.

Each chick was weighed upon arrival and on the last day of each designated week. All of the diet offered to the chicks of each lot was weighed and on the last day of each designated week diet not consumed was weighed back.

On the last day of the designated week, each chick was anesthetized by being placed in a saturated chloroform atmosphere. Blood was obtained from the heart, transferred to citrated centrifuge tubes and plasma separated by centrifugation. Plasma was then obtained, 1.0 ml aliquot from each chick of a lot, pooled to make a total volume of plasma equal to 5.0 ml and stored in glass-stoppered tubes at approximately -18^0 for subsequent vitamin A analysis by the Kimble procedure (23). The liver was then taken, adhering tissue removed, weighed, macerated and stored at aoproximately -18^0 for subsequent vitamin A analysis according to the procedure of Bunnell et al. (24).

Minimum and maximum daily ambient temperature averages with their standard errors were 21 ± 0.3^0 and 26 ± 0.3^0 for the first replicate and 21 ± 0.2^0 and 26 ± 0.3^0 for the second replicate. Natural and incandescent light measured weekly at 1 p.m. averaged for the first replicate 463 ± 30 lux and the second replicate 506 ± 16 lux.

The statistical analysis consisted of computing the variation due to the 10 treatments and due to lots of birds within treatments (error) by analysis of variance according to the procedures of Snedecor and Cochran (25), with weighting of each lot's average response by the number of chicks contained in that lot. The latter was necessary since a few chicks died during the course of the experiment. The variation due to treatment was also subdivided into three additional comparisons, no dietary vitamin A versus plus dietary vitamin A and within lots receiving no dietary vitamin A and within lots receiving dietary vitamin A as follows:

Source of variation	df
(1.) Treatments	9
(2.) Lots within treatments (error)	10
(3.) Total	19

Variation	df
(1.) Treatments	9
(a) (-A) vs (+A)	1
(b) Within (-A)	4
(c) Within (+A)	4

Estimates of rates of loss of vitamin A from the liver were made from regressions of $\log_{10}$ liver vitamin A concentration (as well as $\log_{10}$ total liver vitamin A content and $\log_{10}$ total liver vitamin A content per unit body weight) on experimental days. These regressions were based upon the observation of Frey and Jensen (26) that liver vitamin A loss is proportional to the total amount of liver vitamin A previously present. Thus using $\log_{10}$ liver vitamin A concentration instead of the actual concentration in the regression of this variable on experimental days, loss of liver vitamin A on a percentage basis was computed.

Experiment II. This experiment followed the same procedures as those outlined for Experiment I. Exceptions included an eight day period instead of a two day period prior to providing the basal diet without added vitamin A or the basal diet plus added vitamin A. This longer period was for the purpose of minimizing transfer of vitamin A from the yolk sac to the liver (27) after starting vitamin A depletion and also to possibly provide greater initial liver vitamin A storage. Instead of four experimental weeks only three were included since the fourth experimental week data of Experiment I were not utilized. During the fourth week, diet consumption and growth were reduced in the lots of chicks fed the basal diet only as compared to the same response variables of the lots of chicks fed the basal diet plus vitamin A and thus loss of vitamin A from the liver was less when the growth rate was reduced (16).

Minimum and maximum daily ambient temperatures averaged $20 \pm 0.4^{\circ}$ and $27 \pm 0.6^{\circ}$ for the first replicate and $22 \pm 0.3^{\circ}$ and $28 \pm 0.3^{\circ}$ for the second. Light exposure averaged 388 ± 29 lux and 463 ± 20 lux.

B. WEANLING RABBITS

METHODS for this species have already been published as a Station Research Report (13); see: Experiment I Acute Vitamin A deficiency, and as a Master of Science Thesis (28). Only data for the first eight weeks of the vitamin A depletion period of the rabbits fed the basal diet without added vitamin A are reported herein. This was based upon data reported in Appendix Fig. 1 of the Research Report (13) in which the rate of increase of terminal body weight plateaued after eight weeks.

C. WEANLING RATS

METHODS for this species have already been oublished as a Station Research Report (12); see: Experiment I Acute Vitamin A Deficiency. Only data for the first four weeks of the vitamin A depletion period of the rats fed the vitamin A-free basal diet are reported herein. This was because the rate of increase of terminal body weight, Appendix Fig. I of the Research Report (12), became less for the fifth week.

RESULTS

A. CHICKS

Experiment I. Average diet consumed, terminal body weight and body weight gain for the lots of chicks in Experiment I are given in Table 1. The overall average consumption of the diet was less, $P \leq 0.05$, in the lots of the chicks fed the basal diet with no added vitamin A, and this was primarily attributed to differences occurring in the 28-day groups. Initial body weight per lot averaged 51 g with a SD = 4. The terminal body weights were not subjected to statistical analysis because of the unequal variance between the (-A) and (+A) dietary groups for 21 and 28 days. The average gain in body weight was less, $P \leq 0.05$, in the

lots of chicks fed the basal diet with no added vitamin A and attributable to differences in the observations at 21 and 28 days.

The average plasma carotenoids and vitamin A concentrations and the liver weights are given in Appendix Table II. These will not be considered here, since they are not pertinent to the objectives of the experiment, but may be relevant to other objectives of users of the data presented herein.

Averages of the concentration of vitamin A in the liver, the total content and the total content per unit of body weight are contained in Fig. I. Each x on the graph is the average of two lots of five chicks each except as indicated in footnotes b and c of Table 1. Linear regressions were calculated using the following model:

$$Y = \underset{\pm s_a}{a} + \underset{\pm s_b}{bX}, \quad R^2 = \text{(variation accounted for by the regression)}$$

Regressions of liver vitamin A concentration in $\log_{10}$ μg of vitamin A per gram of liver, Y_- for the groups fed no added dietary vitamin A, and Y_+ for the groups fed added vitamin A on experimental days, X, 0 to 21 day data only, were:

$$Y_- = \underset{\pm 0.06,}{1.40} - \underset{\pm 0.004}{0.128X}, \quad R^2 = 0.94$$

$$Y_+ = \underset{\pm 0.06,}{1.45} + \underset{\pm 0.004}{0.030X}, \quad R^2 = 0.96$$

of $\log_{10}$ total liver vitamin A in μg were:

$$Y_- = \underset{\pm 0.09,}{1.74} - \underset{\pm 0.007}{0.091X}, \quad R^2 = 0.89$$

$$Y_+ = \underset{\pm 0.09,}{1.79} + \underset{\pm 0.007}{0.074X}, \quad R^2 = 0.92$$

and of $\log_{10}$ total liver vitamin A per unit body weight in µg/100 g were:

$$Y_{-} = 1.98 - 0.136X, R^2 = 0.94$$
$$\pm 0.07, \pm 0.005$$
$$Y_{+} = 2.01 + 0.026X, R^2 = 0.74$$
$$\pm 0.07, \pm 0.005$$

Because growth in one lot fed no added vitamin A decreased markedly during the 22-28 days and this in turn was known to decrease vitamin A loss from the liver (14), data from all 28-day groups were not utilized in regression computations. The daily loss of vitamin A from the liver of the lots of chicks fed the basal diet without added vitamin A was 25.5% for concentration, 18.9% for total and 26.9% for total per unit body weight. In contrast the livers of the lots of chicks fed the basal diet plus vitamin A increased slightly. The liver vitamin A concentration of the lots of chicks fed the basal diet without vitamin A decreased to 50% of its initial (0-day) estimated value in 2.3 days; the total amount required 3.3 days; and the amount per unit of body weight 2.2 days.

Carotenoids in the livers of the lots of chicks are contained in Appendix Table III and will not be considered herein.

Experiment II. Diet consumed, terminal body weight, and body weight gain are contained in Table 2. The overall average of diet consumed did not differ between the lots offered the basal diet and the lots offered the basal diet plus vitamin A. Averages were, respectively, 28.0 and 28.7 g/chick/day. Initial body weight per lot in grams averaged 139 with SD = 28. The terminal body weights during the 21-day experimental period were essentially similar for both groups and the overall body weight gains were unaffected by the two diets.

Plasma carotenoids and vitamin A concentrations and liver weights of the chicks are given in Appendix Table IV.

The concentration, total amount and total amount per unit body weight of

vitamin A in livers of the lots of chicks are contained in Fig. 2. Regressions of concentration expressed as $\log_{10}$ µg/g of vitamin A of the lots of chicks offered the basal diet with no vitamin A, Y_-, and of the lots of chicks offered the diet plus vitamin A, Y_+, on experimental days, X, were:

$$Y_- = \underset{\pm 0.04}{1.89} - \underset{\pm 0.003}{0.128}X, \quad R^2 = 0.96$$

$$Y_+ = \underset{\pm 0.04}{1.71} + \underset{\pm 0.003}{0.023}X, \quad R^2 = 0.96$$

of $\log_{10}$ total liver vitamin A in µg were:

$$Y_- = \underset{\pm 0.05}{2.68} - \underset{\pm 0.004}{0.106}X, \quad R^2 = 0.92$$

$$Y_+ = \underset{\pm 0.05}{2.50} + \underset{\pm 0.004}{0.048}X, \quad R^2 = 0.96$$

and of $\log_{10}$ total liver vitamin A per unit body weight in µg/100 g were:

$$Y_- = \underset{\pm 0.03}{2.51} - \underset{\pm 0.002}{0.142}X, \quad R^2 = 0.97$$

$$Y_+ = \underset{\pm 0.03}{2.32} + \underset{\pm 0.002}{0.011}X, \quad R^2 = 0.90$$

The daily loss of vitamin A from the liver of the lots of the chicks offered the basal diet without added vitamin A amounted to 25.5% for concentration, 21.6% for total and 27.9% for total per unit body weight. The livers of the chicks of lots offered the basal diet plus vitamin A increased slightly in vitamin A over the 21 days. The number of days for the concentration of liver vitamin A of lots offered the basal diet without added vitamin A to decrease to 50% of its initial value was 2.3, for the total amount of liver vitamin A 2.8 days and for the total amount of liver vitamin A per unit body weight 2.1 days.

Carotenoids in the livers of the lots of chicks are contained in Appendix Table V.

B. WEANLING RABBITS

Average diet consumed, terminal body weight, and body weight gain are given in Table 3. Cumulative diet consumed decreased slightly with an increase in the number of experimental days and this was reflected in cumulative daily body weight gain. However, terminal body weights increased throughout the 56-day experimental period but the rate of increase was less between 28 and 56 days than between 0 and 28 days.

The vitamin A concentration in the liver as well as the total amount and the total amount per unit of body weight are given in Fig. 3. Regression of liver vitamin A concentration expressed as $\log_{10}$ μg/100 g, $\underline{Y}$, on experimental days, X, was:

$$\underline{Y} = 3.54 - 0.026X, \quad R^2 = 0.68$$
$$\pm 0.21, \quad \pm 0.006$$

of total liver vitamin A as $\log_{10}$ μg was:

$$\underline{Y} = 3.07 - 0.021X, \quad R^2 = 0.56$$
$$\pm 0.17, \quad \pm 0.005$$

and of total liver vitamin A per unit body weight as $\log_{10}$ μg/100 g was:

$$\underline{Y} = 2.10 - 0.029X, \quad R^2 = 0.74$$
$$\pm 0.24, \quad \pm 0.006$$

Daily loss of vitamin A was estimated from the above equations to amount to 5.8% for concentration, 4.7% for total and 6.4% for total per unit of body weight. The number of days for the concentration of vitamin A to decrease to 50% of its initial value was 11.5, for total amount of vitamin A 14.3, and for total amount per unit body weight 10.3 days.

C. WEANLING RATS

Diet consumed, terminal body weight and body weight gain are contained in Table 4. Cumulative diet consumed in g/day increased throughout the 28-day experimental period. The same was true for terminal body weights. Cumulative body weight gain did not vary throughout the experimental period reflecting the slight tendency for diminishing rates of growth; see Appendix Fig. 1 of the Research Report by Frier et al. (12).

The vitamin A concentration in the liver as well as the total amount and the total amount per unit of body weight are given in Fig. 4. Regression of vitamin A concentration in the liver expressed as $\log_{10}$ μg/100 g, Y_-, on experimental days, X, was:

$$Y_- = \underset{\pm 0.09,}{2.88} - \underset{\pm 0.005}{0.087}X, \quad R^2 = 0.88$$

of total liver vitamin A as $\log_{10}$ μg was:

$$Y_- = \underset{\pm 0.08,}{1.46} - \underset{\pm 0.004}{0.074}X, \quad R^2 = 0.87$$

and of total liver vitamin A per unit of body weight as $\log_{10}$ μg/100 g was:

$$Y_- = \underset{\pm 0.09,}{1.56} - \underset{\pm 0.005}{0.091}X, \quad R^2 = 0.89$$

Daily loss of vitamin A from the liver amounted to 18.2% for concentration, 15.7% for total and 18.9% for total per unit of body weight. The time required for concentration of vitamin A to decrease to 50% of its initial value was 3.4, for the total amount 4.1, and for the total amount per unit of body weight 3.3 days.

TABLE 1

Diet consumption, terminal body weight and body weight gain of lots of chicks fed a basal diet without vitamin A (-) and the same basal diet with added vitamin A (+) Experiment I[a]

Criteria	Experimental days 0	7	14	21	28	SD per lot of chicks
Cumulative diet consumed, g/day						
-	0	14	22	26[b]	32[c]	6
+	0	15	23	30[b]	38	
Terminal body weight, g						
-	51	130	280	432[b]	625[c]	-
+	52	143	295	527[b]	744	
Cumulative body weight gain g/day						
-	0	11	16	18[b]	20[c]	5
+	0	13	17	23[b]	25	

a Average of two lots of five chicks each except as indicated

b Four chicks in one lot

c Two chicks in one lot

TABLE 2

Diet consumption, terminal body weight and body weight gain of lots of chicks fed a basal diet without vitamin A (-) and the same basal diet with added vitamin A (+) Experiment II[a]

Criteria	Experimental days 0	7	14	21	SD per lot of chicks
Cumulative diet consumed, g/day					
-	0	30	38	45	6
+	0	31	39	47[b]	
Terminal body weight, g					
-	137	288	520	767	-
+	138	298	526	819[b]	
Cumulative body weight gain, g/day					
-	0	21	27	30	6
+	0	22	28	32[b]	

a Average of two lots of five chicks each except as indicated

b Four chicks in one lot

TABLE 3

Diet consumption, terminal body weight and body weight gain of weanling rabbits fed a vitamin A-free diet[a]

Criteria	Experimental days 0	28	56	SD per rabbit
Rabbits, no	7	8	8	-
Cumulative diet consumed, g/day	0	107	96	20
Terminal body weight, g	826	2098	2504	270
Cumulative body weight gain, g/day	0	39	28	5

a Average of the number of rabbits as indicated in row one

TABLE 4

Diet consumption, terminal body weight and body weight gain of weanling rats fed a vitamin A-free diet[a]

Criteria	Experimental days 0	7	14	21	28	SD per rat
Rats, no	8	11	5	9	10	-
Cumulative diet consumed, g/day	0	10.9	13.1	14.7	15.8	1.9
Terminal body weight, g	74	107	162	191	231	68
Cumulative body weight gain, g/day	0	5.2	5.5	5.6	5.6	1.0

a Averages of the number of rats as indicated in row one

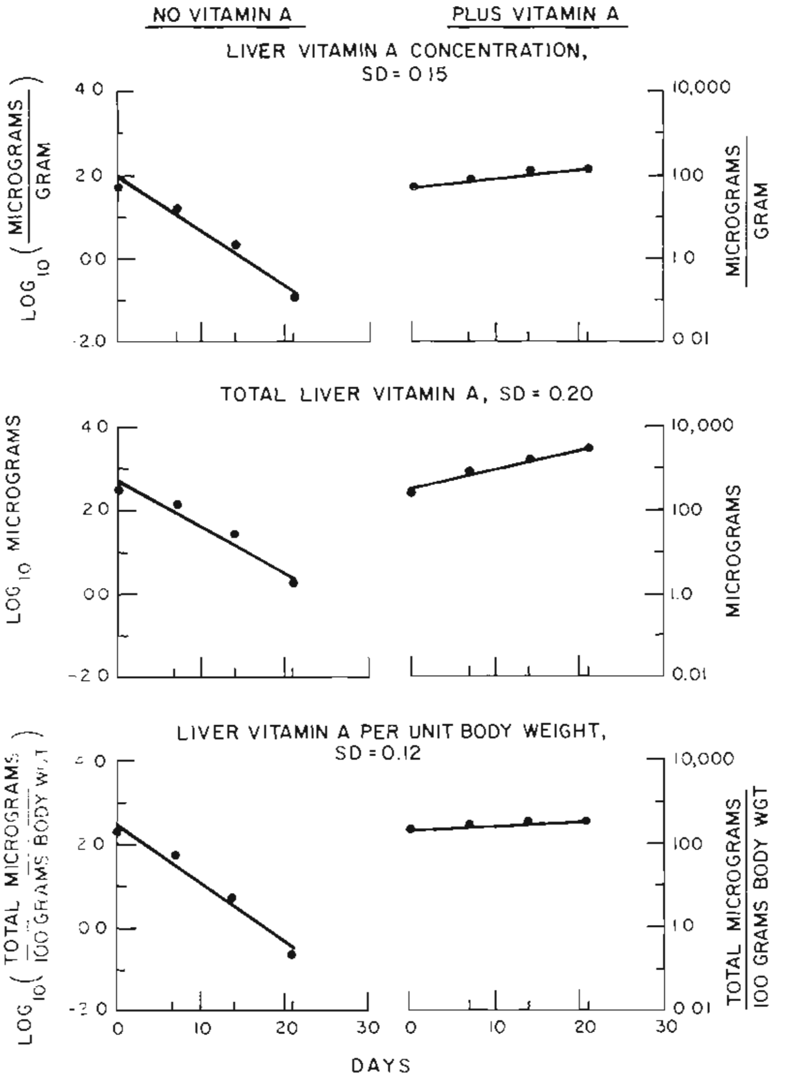

Figure 1.

Rates of loss of liver vitamin A of lots of two-day old male chicks offered ad libitum a basal diet with no added vitamin A and with added vitamin A. Each X on the graph represents the average of two lots of five chicks each. Exceptions to the number of chicks per lot are given in footnotes b and c of Table 1.

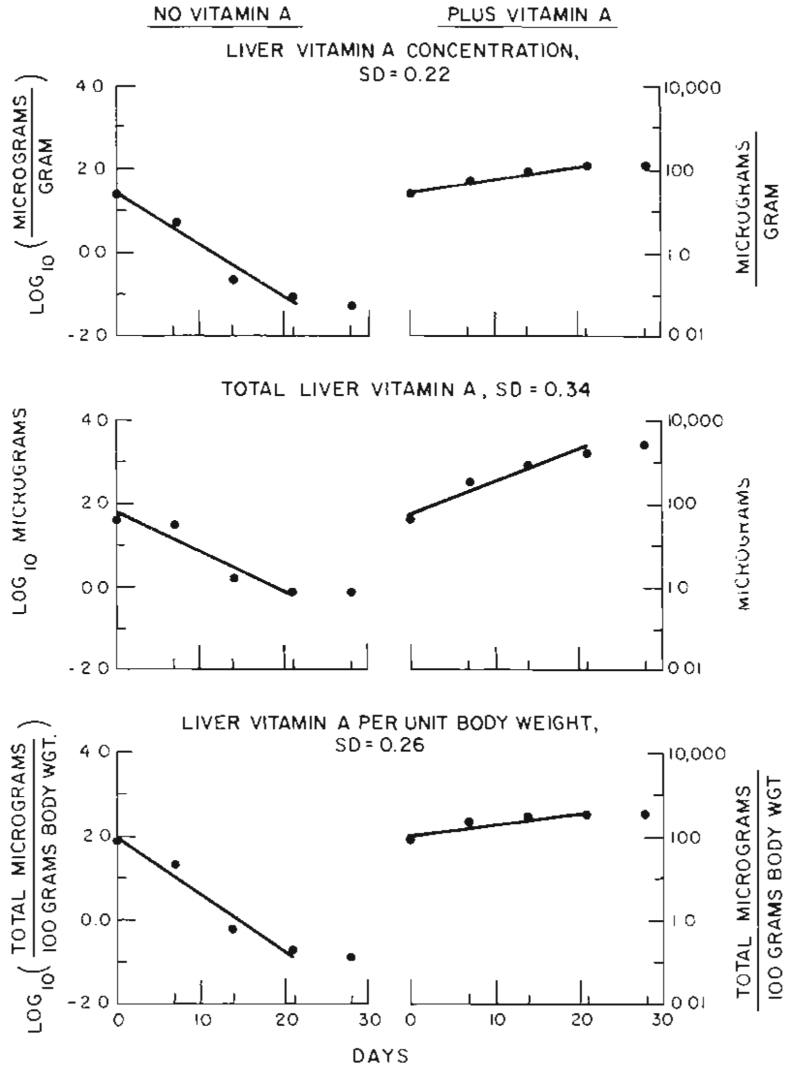

Figure 2.

Rates of loss of liver vitamin A of lots of one-week old male chicks offered ad libitum a basal diet with no added vitamin A and with added vitamin A. Each X on the graph represents the average of two lots of five chicks each. An exception to the number of chicks per lot is given in footnote b of Table 2.

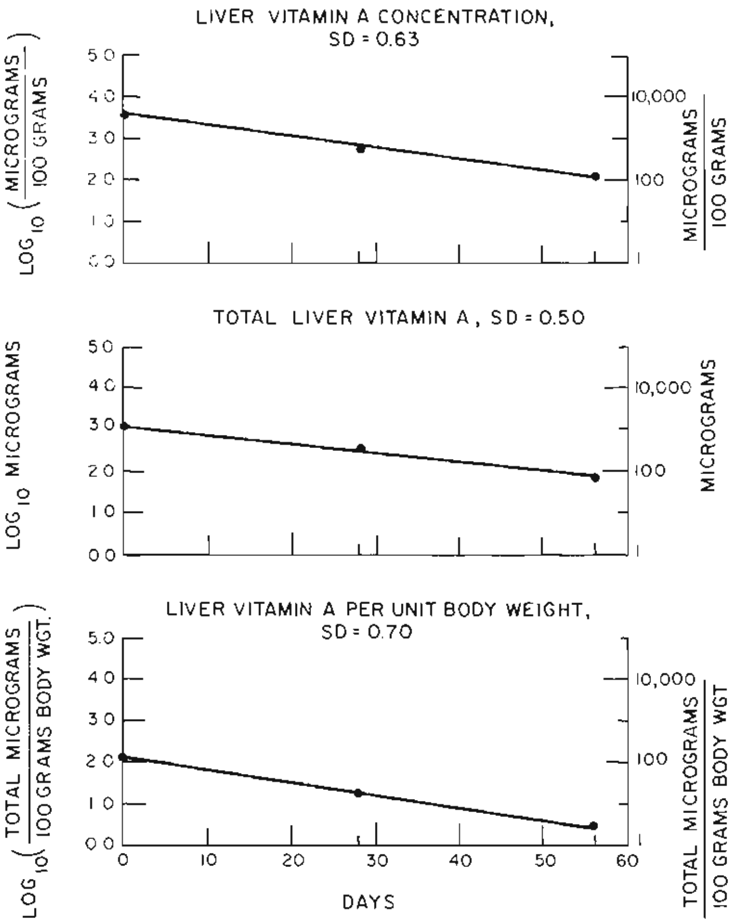

Figure 3.

Rate of loss of liver vitamin A of weanling male five-week old rabbits offered ad libitum a vitamin A-free basal diet. Each X on the graph represents the average of seven rabbits for the 0-day and eight rabbits each for the 28th and 56th days.

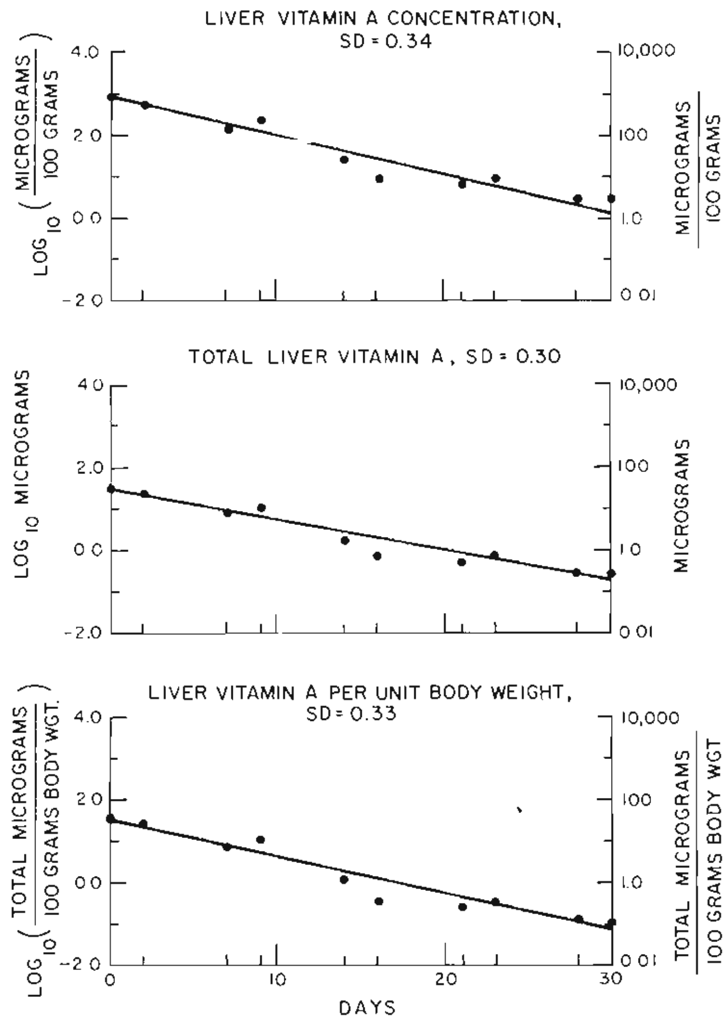

Figure 4.

Rate of loss of liver vitamin A of weanling male four-week old rats offered ad libitum a vitamin A-free basal diet. Each X on the graph represents the average of 6, 2, 6, 5, 4, 1, 5, 4, 7 and 3 rats for the 0, 2, 7, 9, 14, 16, 21, 23, 28 and 30 days, respectively.

DISCUSSION

The estimates of rate of loss of vitamin A from the liver of chicks are in reasonable agreement with some of the previous literature - see Appendix Table VI. For example, new born chicks with initial stores of 75, 310 and 430 I.U. of vitamin A per liver were estimated to require, respectively, 3.6, 2.5 and 2.9 days to deplete their liver stores to 50% of their initial value. These estimates were calculated in a similar manner to those reported herein and were based upon the data of lots 1, 5 and 9 of Table 1 of the publication of Bolin et al. (29). Similar estimates of the lots of chicks of the present experiment were 3.3 for Experiment I and 2.8 days for Exoeriment II, values which agree reasonably well with those of Bolin et al. (29). In possible contrast was the report of Braekkan (30) in which 14-day old chicks were administered five graded levels of vitamin A, 250, 1,000, 4,000, 16,000 and 61,000 I.U., in a single dose. Thereafter the chicks were offered a vitamin A deficient diet. Braekkan (30) estimated the loss of vitamin A to be 10, 20, 60 and 100 I.U./day from the liver of the chicks administered respectively, 250, 1,000, 4,000 and 16,000 I.U. of vitamin A. Using average values as oresented in Tables 1-4, our linear regression estimates in I.U./day, were 9.9, 22.4, 60.0 and 96.2. The livers of the group administered the highest dose, 61,000 I.U., exhibited no trend in loss of vitamin A until after the 40th post-dosage day. Calculations similar to those reported herein for the time required for the total amount of vitamin A in the liver to decrease to 50% of its initial value were for 250, 1,000, 4,000 and 16,000 I.U. groups as follows: 3.6, 12.3, 20.3 and 37.0 days. Thus absolute loss, I.U./day, was roughly proportional to the initial liver storage of vitamin A and the percentage loss was inversely related to the initial stores. Braekkan (30) stated that the loss of vitamin

A from the liver of chicks was not proportional to that already present as proposed by Hickman and as cited and confirmed by Frey and Jensen (5) with steers. However the R^2 values of regressions of actual liver vitamin A concentration on experimental days were not too different from those values of regressions of $\log_{10}$ liver vitamin A concentration on experimental days. The R^2 values for the two types of regressions, actual amount versus $\log_{10}$ amount on days were in order of increasing dosage of vitamin A, respectively, 0.94, 0.90; 0.81, 0.84; 0.98, 0.91 and 0.98, 0.92.

No values on liver vitamin A content of rabbits maintained on vitamin A-free diets over varying time periods were readily found in the literature. Therefore no comments can be made with respect to the estimated values contained herein.

Considerable data existed on rats, Baumann et al. in 1934 (31), Davies and Moore, 1935 (32) and 1937 (33), Brenner, 1942 (34), High, 1954 (35), and McGillivray, 1961 (36), and were suitable for calculations similar to those utilized for the data from the experiments contained herein - see Appendix Table VII. In 28 rats, weight 70 - 110 grams and fed halibut liver oil for eight days followed by a low vitamin A diet, the time to deplete total liver stores was estimated by regression of $\log_{10}$ total blue units on experimental days offered the low vitamin A diet to be 32.6 days, Table 1 of reference (31). In 18 month old pie-bald rats, Table 1 reference (32), concentration of vitamin A in the liver, B.U. per gram, decreased to 50% in 16.9 days. In a similar study by the same authors, (33) Fig. 1, but with 50 - 75 g rats and lower initial values, total I.U., 25.8 days were required to deplete livers to 50% of their initial vitamin A content. In male rats weighing on the average 192 g, data reported by Brenner (34) in Table 2, the time required to deplete concentration to 50% of its initial value was 13.2 days, total amount 13.6 days and

total amount per unit of body weight 11.5 days. High (35) provided data, Table 3, of two groups of rats, 4 - 5 weeks old and 3 - 4 weeks old, with differing initial liver stores of 625 mg and 62 mg. Time required to deplete to 50% of original value was calculated to be 8.8 and 9.6 days. In the case of the data of the rats with low initial stores there was evidence of significant $P \leq 0.01$, negative quadratic curvature, a finding not present in any of the other cited rat experiments. Lastly, McGillivray (36) depleted rats with massive initial stores of the vitamin, 34,500 mg/liver. The 50% depletion time was calculated to be 28.7 days. In addition, Sundaresen (37) in 1977 determined the half-life of a single dose of ^{14}C-labelled retinol in weanling male rats maintained on retinoic acid. A rapidly declining pool of 0.75 days, a slowly declining pool with a constant rate of decrease, and a pool with a half-life of 13 days which began approximately 6 weeks after dosing were reported. Regression of loss of total liver vitamin expressed as $\log_{10}$ µg, Y, on days X, after dose was $Y = 2.48 - 0.0498X$ with the "one-half life" for total liver vitamin A content equal to 7 days. Since retinoic acid may decrease the mobilization of vitamin A from the liver (19), these reported half-lives of the tracer may be overestimated. In summary the estimates of the time required to deplete total liver stores varied from 8.8 to 32.6 days. Because of the variation in age, sex, initial body weight, body weight gain, diet and possibly of storage of the vitamin prior to depletion, these data are difficult to evaluate. It would appear that based on the estimates of the data from the rats of the study reported herein and of the data reported by Sundaresen (37), the time to deplete the total liver storage of vitamin A to 50% of its initial amount in weanling rats with moderate initial storage, fed diets adequate with respect to contemporary dietary requirements and growing rapidly is probably less than the values obtained from the data reported by most of the earlier researchers

during the period 1934 - 1961.

It was of interest to compare the estimates of depletion rates across species of those utilized in the present experiments and of those available in the literature. Unfortunately in the case of those estimates available in the literature only one on weanling pigs (11) provided adequate data to make estimates and were under dietary conditions which provided no vitamin A or its precursor, carotenoids. In the often cited study of Frey and Jensen (26) the basal diet contained carotene, see Table 2 of reference (5). This was equally so with respect to similar studies with dairy cattle (6), chicks (7), feedlot cattle (8) and sheep (9). In the case of the weanling pigs which weighed initially 14.1 kg and had body weight gains of 0.7 kg/day, Table 4 of reference (11), the linear regression (weighted according to the number of pigs per group) for the loss of liver vitamin A expressed as $\log_{10}$ total μg, Y, on depletion days, X, equalled: $Y = 3.71 - 0.0204X$ with an $R^2 = 0.99$. The daily loss amounted to 4.6%/day and the number of days to deplete to 50% of initial stores was 14.7 days. The estimates of these weanling pigs and those of the chick, rabbit and rat reported herein are contained in Table 5. In terms of both daily depletion rate of liver vitamin A storage and time required to deplete to 50% of initial storage, it appeared that the first was inversely related to initial body weight of the species and the second directly related to the initial body mass of the species. Simple correlations, for example, between initial body weight and daily liver vitamin A depletion rate and initial body weight and days required to deplete to 50% of the initial liver vitamin A content were, respectively, -0.66 and +0.70 with significance levels of $P \leq 0.30$ and $P \leq 0.20$. Thus considerably more data are required before any certainty can be ascertained for the relationships in depletion rates among

species. Also, since within a species of individuals of comparable body weight, there appears to be a relationship between rate of loss and initial stores of the vitamin (30), there may not be a single rate of depletion.

TABLE 5

Rates of liver vitamin A depletion of the chick and weanling rat, rabbit and pig[a]

Species	Initial body weight	Daily gain in body weight	Daily liver vitamin A depletion rate	Time required to deplete the initial storage to 50%
	(g)	(g/day)	(%)	(days)
Chicks				
Exp. I	51	11-20	18.9	3.3
Exp. II	137	21-30	21.6	2.8
Rabbits	826	28-39	4.7	14.3
Rats	74	5-6	15.7	4.1
Pigs				
(Ref. 11)	14,000	700	4.6	14.7

a All data except for pigs (11) were obtained from experiments reported herein

SUMMARY

Two-day old and eight-day old male chicks, weanling male rabbits and weanling male rats were placed on vitamin A-free diets for varying periods of time to ascertain the rate of depletion of liver vitamin A stores. The daily rate of loss of liver vitamin A stores amounted to 18.9% and 21.6% for the two groups of chicks, 4.7% for the rabbits and 15.7% for the rats. The times required to deplete the liver stores to 50% of initial values were, respectively, 3.3, 2.8, 14.3 and 4.1 days. When comparing these results and those available from the literature for the weanling pig, there appeared to be an inverse relationship between initial body weight and rate of loss and a positive relationship between initial body weight and the time required to deplete to 50% of the initial stores. However such comparisons may be limited, since in at least one species, the chick, it was reported that the rates of loss were affected by the initial stores of the vitamin. Hickman's proposition as cited by Frey and Jensen (26) that the loss of vitamin A is proportional to the reserves at that time was supported by the chick, rabbit and rat data of the experiments reported herein, as well as by computations of data available from the literature for day-old chicks, rats and pigs, except for one rat study (35). However, when rates of depletion of groups of 14-day old chicks with widely varying initial stores of the vitamin (30) were calculated, the percentage rate of loss was found to vary inversely with the level of initial storage. Thus, level of initial storage may well have to be considered as another factor among the many affecting rates of depletion of liver vitamin A stores.

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APPENDIX TABLE I

Chick vitamin A-free basal ration[1]

Ingredients	kg/100 kg
Casein, vitamin free test[2]	15.000000
Soybean, seeds wo hulls, solv extd, grnd, mx 3% fiber	15.000000
Corn, white, grain, grnd[3]	62.711091
d,l-methionine	0.600000
l-Arginine HCl	0.200000
Cottonseed oil[2]	2.870000
Mineral mix[4]	3.412760
Vitamin mix[5]	0.006149
Choline premix[6]	0.200000
	100.000000 kg

1. Formulated to meet the chick's minimum NAS-NRC, 1971, nutrient requirement except vitamin A and based essentially on the ration of Pudelkiewicz et al., Poul. Sci., 43:1157, 1964

2. Supplied by General Biochemicals, Chagrin Falls, Ohio

3. Supplied by Quaker Oats Co.

4. Contains: 1600g $CaHPO_4$; 1000g $CaCO_3$; 510g $MgSO_4 \cdot 7H_2O$; 250g NaCl; 26.6g $FeC_6H_5O_7 \cdot XH_2O$ (16% Fe); 15g $MnSO_4 \cdot H_2O$; 10g $ZnCO_3$; 1.10g $CuSO_4 \cdot 5H_2O$; 0.060g $NaIO_3$
Supplied by J.T. Baker Co.

5. Contains: 60mg vitamin K_3 (menadione); 2000mg (1000 IU) Roche vitamin E (50% dl-α-tocopheryl acetate beadlets); 1400mg folacin (3%); 150mg niacin; 900mg vitamin B_{12} (0.1%); 700mg vitamin D (30,000 ICU/g); 400mg Ca-pantothenate; 400mg pyridoxine-HCl; 130mg riboflavin; 9mg biotin
Supplied by General Biochemicals, Chagrin Falls, Ohio

6. Choline premix contains: 100g choline chloride; 92.6ml water; 7.4ml sodium selenite solution (1.357 mg/ml)

APPENDIX TABLE II

Plasma carotenoids, plasma vitamin A and liver weights of lots of chicks fed a basal diet without (-) and with (+) vitamin A - Experiment I[a]

Criteria	Experimental days 0	7	14	21	28	SD per lot of chicks
Plasma carotenoids, μg/100 ml						
-	2,726	244	52	38[b]	27[c]	-
+	2,726	260	74	37[b]	24	
Plasma vitamin A, μg/100 ml						
-	179	42	8	6[b]	4[c]	-
+	179	102	94	103[b]	94	
Liver weight, g						
-	1.7	5.7	8.6	10.4[b]	15.6[c]	-
+	1.6	6.6	9.5	14.3[b]	18.6	
Weight per unit body weight, g/100 g						
-	3.3	4.3	3.0	2.4[b]	2.4[c]	0.7
+	3.0	4.7	3.2	2.7[b]	2.5	

a Weighted means of two lots of chicks per treatment, with five chicks per lot except as indicated

b Four chicks per lot in one lot

c Two chicks per lot in one lot

APPENDIX TABLE III

Carotenoids in livers of lots of chicks fed a basal diet without (-) and with (+) vitamin A - Experiment I[a]

Criteria		Experimental days 0	7	14	21	28	SD per lot of chicks
Concentration, μg/g							
Actual	-	47.4	6.5	1.6	1.0[b]	0.4[c]	-
	+	52.4	6.4	1.8	0.7[b]	0.6	
Log_{10}	-	1.64	0.79	0.20	-0.02[b]	-0.35[c]	0.15
	+	1.71	0.78	0.24	-0.16[b]	-0.27	
Total, μg							
Actual	-	76	38	14	10[b]	7[c]	-
	+	83	41	17	10[b]	10	
Log_{10}	-	1.86	1.54	1.13	0.98[b]	0.81[c]	0.23
	+	1.91	1.60	1.21	0.99[b]	1.00	
Total per unit body weight, μg/100 g							
Actual	-	149	28	5	2[b]	1[c]	-
	+	158	30	6	2[b]	1	
Log_{10}	-	2.16	1.42	0.68	0.35[b]	0.02[c]	0.19
	+	2.20	1.46	0.72	0.27[b]	0.12	

a Weighted means of two lots of chicks per treatment, with five chicks per lot except as indicated

b Four chicks per lot in one lot

c Two chicks per lot in one lot

APPENDIX TABLE IV

Plasma carotenoids, plasma vitamin A and liver weights of lots of chicks fed a basal diet without (-) and with (+) vitamin A - Experiment II[a]

Criteria	Experimental days 0	7	14	21	SD per lot of chicks
Plasma carotenoids, μg/100 ml					
-	273	58	30	20	-
+	254	70	36	26[b]	
Plasma vitamin A, μg/100 ml					
-	105	58	37	7	-
+	102	100	109	102[b]	
Liver weight, g					
-	5.7	9.5	13.9	16.4	-
+	5.6	10.2	13.9	19.0[b]	
Weight per unit body weight, g/100 g					
-	4.2	3.3	2.7	2.1	0.7
+	4.0	3.4	2.6	2.3[b]	

a Weighted means of two lots of chicks per treatment, with five chicks per lot except as indicated

b Four chicks per lot in one lot

APPENDIX TABLE V

Carotenoids in livers of lots of chicks fed a basal diet without (-) and with (+) vitamin A - Experiment II[a]

Criteria		Experimental days 0	7	14	21	SD per lot of chicks
Concentration, μg/g						
Actual	-	6.4	1.4	0.8	0.5	-
	+	6.6	1.9	0.6	0.4[b]	
Log_{10}	-	0.78	0.14	-0.14	-0.29	0.14
	+	0.79	0.25	-0.24	-0.34[b]	
Total, μg						
Actual	-	36	14	11	9	-
	+	37	19	8	9[b]	
Log_{10}	-	1.54	1.12	1.00	0.92	0.17
	+	1.54	1.26	0.90	0.93[b]	
Total per unit body weight, μg/100 g						
Actual	-	26	5	2	1	-
	+	27	6	2	1[b]	
Log_{10}	-	1.40	0.66	0.25	0.04	0.11
	+	1.39	0.78	0.18	0.02[b]	

a Weighted means of two lots of chicks per treatment, with five chicks per lot except as indicated

b Four chicks per lot in one lot

APPENDIX TABLE VI

Regressions of $\log_{10}$ liver vitamin A, Y, on days, X, fed a vitamin A-free diet - chicks

Author(s)	Regressions	Literature reference
Bolin et al.		Table 1 (29)
Total I.U.		
Lot 1	$Y = 1.96 - 0.0820X$, $R^2 = 0.89$	
Lot 5	$Y = 2.58 - 0.1177X$, $R^2 = 0.95$	
Lot 9	$Y = 2.64 - 0.1047X$, $R^2 = 0.97$	
Braekkan		Tables 1-4 (30)
Total I.U.		
250 I.U. dose[a]	$Y = 2.01 - 0.1528X$, $R^2 = 0.90$	
	($Y = 71 - 9.94X$, $R^2 = 0.94$)[e]	
1,000 I.U. dose[b]	$Y = 2.76 - 0.0276X$, $R^2 = 0.84$	
	($Y = 549 - 22.38X$, $R^2 = 0.81$)[e]	
4,000 I.U. dose[c]	$Y = 3.55 - 0.0342X$, $R^2 = 0.91$	
	($Y = 2434 - 59.96X$, $R^2 = 0.98$)[e]	
16,000 I.U. dose[d]	$Y = 4.03 - 0.0220X$, $R^2 = 0.92$	
	($Y = 7155 - 96.62X$, $R^2 = 0.98$)[e]	

a Means 24 hrs through 6 days utilized in regressions

b Means 2 through 16 days utilized in regressions

c Means 3 through 40 days utilized in regressions

d Means 3 through 76 days utilized in regressions

e Actual total I.U. utilized in regressions instead of $\log_{10}$ total I.U.

APPENDIX TABLE VII

Regressions of $\log_{10}$ liver vitamin A, Y, on days, X, fed a vitamin A-free diet - rats

Author(s)	Regressions	Literature reference
Baumann et al.		Table 1 (31)
Total blue units	$Y = 3.36 - 0.0092X$, $R^2 = 0.91$[a]	
Davies and Moore		Table 1 (32)
Total blue units	$Y = 4.14 - 0.1242X$, $R^2 = 0.84$	
Davies and Moore		Fig. 1 (33)
Total I.U.	$Y = 3.29 - 0.0116X$, $R^2 = 0.77$	
Brenner[b]		Table 2 (34)
Units/100g liver	$Y = 4.02 - 0.0228X$, $R^2 = 0.89$	
Total units	$Y = 2.99 - 0.0221X$, $R^2 = 0.88$	
Total units/100g B.W.	$Y = 2.71 - 0.0261X$, $R^2 = 0.94$	
High		Table III (35)
Total µg		
Series 1	$Y = 2.73 - 0.0342X$, $R^2 = 0.95$[a]	
Series 2	$Y = 2.02 - 0.0312X$, $R^2 = 0.86$[a,c]	
McGillivray		Table 5 (36)
Total µg	$Y = 4.62 - 0.0731X$, $R^2 = 0.76$[a]	

a Weighted regression according to number of rats per group

b Only data from male rats for 56 days (8-weeks) utilized in regression calculations

c Statistically significant, $P \leq 0.01$, negative quadratic curvature present