A STUDY OF NUTRIENT CONDITIONING. UTILIZATION OF BETA CAROTENE OF NATURAL SOURCES BY CHICKS PREVIOUSLY GIVEN VITAMIN A per se

by

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INTRODUCTION

Many research workers have observed that animals fed a particular diet for a certain length of time show reactions that are considered to be characteristic effects of the feeding. This implies that when the feeding time is long enough, the nature of the biological system in the animal changes in response to the administered nutrients. The phenomenon is a case of adaptation.

Not much is understood about the mechanism of adaptation in biochemical terms. Participation of hormones and enzymes in the process has been postulated in certain studies.

Physiologists recognize conditioning effects such as the adaptation syndrome, immunization to diseases or resistance to drugs. Nutritionists relate conditioning to metabolic changes within the organism under study, initiated by variation of diet composition or dietary level of nutrients. The parameter used in nutrient adaptation studies depends on the characteristics of the administered substance.

By adaptation is meant the response of the body system to a stress in general. Response to a change of dietary level or type of diet is nutrient adaptation. The term nutrient conditioning is used in this work to refer to the altered response of the body system to a nutrient fed following the prior feeding of a different but related nutrient for a certain length of time.

It is questionable whether prefeeding of a certain nutrient affects the utilization of nutrients subsequently fed. In this work an attempt was made to determine whether chicks could be conditioned to the use of pure vitamin A so that after a switch to beta-carotene as the source of vitamin A activity they would not utilize it as well as without the prefeeding of pure vitamin A.

The experiment was designed to observe how chicks would utilize beta-carotene from alfalfa as a vitamin A source, compared to the utilization of pure vitamin A, after they had been fed 1000 International Units (IU) pure vitamin A per kg of feed for a prior 4-week period. Performance was evaluated in terms of vitamin A content of liver and serum of the chicks. Growth and feed conversion ratio were determined to provide supporting information. This investigation was based on four experiments carried out at different times.

REVIEW OF LITERATURE

Knox et al. (1) described adaptation as a metabolic process that potentially altered the pattern of metabolism by altering the nature of the metabolic machinery in the cell. Mitchell (2) proposed that digestive enzymes act as metabolic regulators to adjust the animals to changes in food consumption. The enzymes might also be influenced by metabolites such as the activating action of trihydroxy bile acids on pancreatic cholesterol esterase (3). Carroll (4) studied the conditioning effect of subsequent feeding of varied carbohydrate and fat sources upon glycolysis and lipogenesis in the rat. Based on her observations she proposed a tentative explanation supporting the hypothesis that by altering the type of dietary fat the responses of glucose-6-phosphatase and fructose diphosphatase enzyme systems to changes in the type of carbohydrate fed could be modified.

According to Selye (5) adaptation to any stimulus is always acquired at the cost of adaptation energy. The experiment described was not in the field of nutrition but it gave some information on adaptation phenomena.

Thymus atrophy or decrease in the weight of the thymus of the rat was used as index of damage caused by stimuli. It was found that during adaptation to a certain stimulus the resistance to other stimuli decreased. The stimuli were drug injections. Effects of drug injection subsequent to the injection of either the same or a different drug, or without pretreatment were compared.

Many studies have been performed to relate dietary changes to state of resistance against disease and infection (6). Squibb et al. (7) reported that chicks that were conditioned from day of hatch with diets containing abnormal quantities of protein and L-lysine showed a greater degree of tuberculous involvement than those fed diets with the normal amounts of these nutrients. In working with mice, Hedgecock (8) found that the resistance to tuberculous infection was increased by dietary administration of a group of fatty acid esters in the diet. He also reported on the conditioning effect of dietary level of protein. With a fatty acid mixture incorporated in the diet, resistance to tuberculosis was greatest in groups of mice that had received rations containing 20% protein. The resistance was not altered by variation in the protein content of the diet from 10 to 40% when lard or methyl linoleate was the sole source of dietary lipid. Other reports confirming the relationship between nutrition and resistance to infection have been given by Howie and Porter (9), and by Schneider (10). Somewhat similar to these findings is a report by Kornegay et al. (11) that urease immunization was effective in stimulating antibody production.

Experiments have been performed by Kaufman et al. (12), Olson and Vester (13), and Savage and Goldstone (14) to study the effect of nutrients on serum lipid levels, particularly the serum cholesterol levels. It was

mentioned (3) that according to Public Health surveys a statistical correlation exists between the incidence of coronary heart attacks and serum cholesterol levels; those with the lower levels are less susceptable. Rademeyer and Booyens (15) proposed a hypocholesterolaemic effect of maize feeding, possibly attributed to its high fiber content. According to Antonis and Bersohn (16), and McGandy et al. (17) effects of carbohydrate and fiber on serum cholesterol are generally of a much smaller order of magnitude than effects of fat.

There are cases in which the effect of nutrients appears to be a type of inhibition, such as the effect of unheated soybean meal on fat absorption as reported by Nesheim et al. (18) and by Garlich and Nesheim (19). Mohrhauer and Holman (20), and Rahm and Holman (21) demonstrated that linoleic acid can inhibit the transformation of linolenic acid into its derivative acids and vice versa. Likewise Lowry and Tinsley (22) observed an inhibition effect of oleic acid on linoleate metabolism in the liver tissue of rats.

Adaptation phenomena thus far reviewed are related especially to type of diet. Another aspect would be adaptation that is initiated by changes in food supply. Animals subjected to nutritional stress, such as inadequate or an excessive supply of one or more of the essential nutrients, might react in such a way as to minimize the effects of nutritional stress. This was discussed in a broad sense by Mitchell (23) with special respect to the ability of the human body to adapt itself in case of restricted food supply. Hill et al. (24) discussed adaptation phenomenon in relationship to the increased capacity of rat livers to convert acetate carbon to fatty acids when they were fed diets devoid of fat. Wertheimer and Ben-Tor (25)

concluded that rats adapted to a limited undernutrition showed a temporary increase in deposition of glycogen and fat though their diet was deficient in carbohydrate.

Several research workers have reported on the effect of a prefed diet upon the metabolism of the substance that was administered subsequently by other than the oral route. Ganther et al. (27) reported that rats injected with single subacute doses of selenite, volatilized selenium in amounts that depended upon the diet fed previously. Based on these and other findings by Ganther and Baumann (28, 29), Hopkins et al. (30) conducted a tracer study on rats. The animals were injected with microgram quantities of radioselenium following the prefeeding of one of 3 types of low selenium diets for 2 weeks. It was observed that both the amounts of radioselenite retained in the carcass and the amounts excreted in the urine depended upon the level of selenium that had been added to the diet fed previously. Teree et al. (31) investigated the effects of radiostrontium when injected intraperitoneally after rats were fed stable strontium mixed with the diet in the form of strontium lactate. Reduced strontium retention was observed which might be contributed to an adaptive excretory process generated by high strontium prefeeding. However, the authors regarded this as an unsupported hypothesis. These studies cannot be considered true nutritional adaptation since parenteral administration was used.

Few reports have been found on the influence of prefed nutrients upon the utilization of those subsequently fed. An experiment carried out by Fisher et al. (32) is one example of the particular type of nutrient adaptation to be studied in this work. They reported, based on a series of experiments with male college students, that there was an inverse

relationship between prior protein intake and the subsequent utilization of high N- low tryptophan diets.

PROCEDURES

Care and Treatment of Experimental Animals

Newly hatched Hyline cockerels[1] were used as experimental animals. The birds were wing banded, weighed individually, and housed in wire-floored batteries with controlled temperature and light.[2] They were placed on a vitamin A deficient diet (table 1) for 7 days to deplete them of most of their initial vitamin A stores. Feed and fresh water were given ad libitum.

When the animals were fed different diets later in the experiment, records of quantities of feed consumed were kept. Weights of the birds were recorded each week. Feed utilization and growth data were used for the determination of feed conversion ratio, the ratio of amount of feed consumed to increase in body weight.

After the 7-day depletion period, chicks received 1000 IU vitamin A per kg of basal diet for 4 weeks, However, one group of chicks remained on the vitamin A deficient diet as a negative control. After being fed the diet supplemented with 1000 IU vitamin A per kg, for 4 weeks chicks were distributed at random into test groups, each group receiving different vitamin A supplements for an additional 2 weeks. At certain intervals during the experiment some chicks were sacrificed and the sera and livers were saved for vitamin A assay.

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[1]Obtained from a commercial hatchery in Topeka, Kansas.

[2]Temperature was controlled thermostatically; light-dark cycle was controlled by a timer device.

TABLE 1

Composition of vitamin A deficient basal diet

Ingredients	Grams
Corn, white, ground	12,217
Soybean oil meal, 44% solvent extracted	5,882
Dried skim milk	407
Brewer's dried yeast	407
Salt	90
Steamed bone meal	407
Calcium carbonate	204
Wesson oil	136
Mineral premix	68
Vitamin premix	182
Total	20,000

Composition of mineral premix per 100 g: $MnSO_4.H_2O$, 16.6 g and wheat middlings, 83.3 g.

Composition of vitamin premix per 100 g: menadione, 0.0058 mg; vitamin D_3 (15,000 ICU/g)[1], 1.0 g; Proferm 20 (B_{12})[2], 4.5 g; DL-methionine, 11.5 g; Merck 1233[3], 1.5 g; choline chloride, 3.0 g; wheat middlings, 160 g.

[1]International Chick Units.

[2]Commercial Solvents Corporation, New York. Vit B_{12} content, 20 g/lb.

[3]Merck and Co. Composition per lb: riboflavin, 8,000 mg; D-pantothenate, 19,720 mg; niacin, 29,000 mg; choline chloride, 80,000 mg.

Sources of vitamin A activity which were used in these experiments, were ANRC[3] vitamin A standard and dehydrated alfalfa meal[4], the activity of which was determined prior to each individual experiment. Both ANRC vitamin A standard and alfalfa meal were stored at -20° C.

Collection and Preparation of Samples of Serum and Liver for Analysis

Chicks were sacrificed and blood was collected in 50 ml centrifuge tubes. The livers were weighed individually and stored in capped bottles, each identified with the wing band that had been attached to the chicks. The livers were stored at -20° C until used for analysis.

Blood from 2 or 3 chicks was pooled. The blood was allowed to clot for about 30 minutes and centrifuged at 2,000 - 3,000 r.p.m. in 2 steps. First, for 15 minutes, after which the clot was cut into 4 longitudinal sections to allow better serum separation, and again centrifuged for an additional 15 minutes. Pooling of samples was necessary to obtain sufficient serum for analysis. Sera were stored at 5° C until used for assay of vitamin A.

Outlines of the Four Experiments

Outlines of the design of each of the 4 experiments are given in tables 2 - 5.

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[3]Animal Nutrition Research Council. A gelatin beadlet type stabilized product, obtained from United States Pharmaceutical (USP), New York. Beadlets were made to approximately 10,000 IU per g, with actual potency supplied with each batch of standard. Potency of product used in formulation of diets was 11,600 IU per g.

[4]A commercial product by courtesy of Manhattan Milling Co., Manhattan, Kansas; selected for high quality.

TABLE 2

Outline of Experiment I

Experimental period (week)	Group number	Type of diet and number of chicks at end of week	No. of surviving negative controls at end of week
0[1]	0[2]	-	-
1	0	depletion diet (63)[3]	10
2	0	1000 ANRC[4] (62)	10
3	0	1000 ANRC (61)	9
4	0	1000 ANRC (61)	5
5	0	1000 ANRC (61)	0
10 chicks sacrificed[5] at the end of the fifth week			
6	I	depletion diet (8)	
	II	1000 alfalfa[6] (9)	
	III	2000 alfalfa (8)	
	IV	3000 alfalfa (9)	
	V	4000 alfalfa (9)	
	VI	1000 ANRC (8)	
4 chicks from each group sacrificed[7] at the end of the sixth week			

[1]Start of experiment; 74 newly hatched chicks, 10 were used for negative controls.

[2]All chicks received same treatment.

[3]Number of chicks still living in parentheses; 1 chick died during depletion period, and 2 chicks (group 0) died during the second and third week.

[4]Units of vitamin A potency per kg of feed supplied by ANRC vitamin A standard.

[5]Chicks were sacrificed for vitamin A assay of the sera and livers; remaining chicks randomized (8 or 9/group) into groups I - VI.

[6]Units of vitamin A potency per kg of feed supplied by alfalfa meal.

[7]Chicks were sacrificed for vitamin A assay of the sera and livers.

TABLE 3

Outline of experiment II

Experimental period (week)	Group number	Type of diet and number of chicks at end of week	No. of surviving negative controls at end of week
0[1]	0[2]	-	-
1	0	depletion diet (90)[3]	16
2	0	1000 ANRC[4] (90)	16
3	0	1000 ANRC (90)	15
4	0	1000 ANRC (90)	11
5	0	1000 ANRC (90)	5
10 chicks sacrificed[5] at the end of the fifth week			
6	I	depletion diet (11)	0
	II	1000 alfalfa[6] (12)	
	III	2000 alfalfa (11)	
	IV	500 alfalfa & 500 ANRC (12)	
	V	1000 Alfalfa & 1000 ANRC (11)	
	VI	4000 alfalfa (12)	
	VII	1000 ANRC (11)	
4 chicks from groups I, II, VI and VII, and 5 from groups III, IV and V sacrificed[7] at the end of the sixth week			
7	I	depletion diet (7)	
	II	1000 alfalfa (8)	
	III	2000 alfalfa (6)	
	IV	500 alfalfa & 500 ANRC (7)	
	V	1000 alfalfa & 1000 ANRC (6)	
	VI	4000 alfalfa (8)	
	VII	1000 ANRC (7)	
4 chicks from each group sacrificed[7] at the end of the seventh week			

[1]Start of experiment; 106 newly hatched chicks, 16 were used for negative controls.

[2]All chicks received same treatment.

[3]Number of chicks still living in parentheses.

[4]Units of vitamin A potency per kg of feed supplied by ANRC vitamin A standard.

[5]Chicks were sacrificed for vitamin A assay of the sera and livers; remaining chicks randomized (11 or 12/group) into groups I - VII.

[6]Units of vitamin A potency per kg of feed supplied by alfalfa meal.

[7]Chicks were sacrificed for vitamin A assay of the sera and livers.

TABLE 4

Outline of experiment III

Experimental period (week)	Group number	Type of diet and number of chicks at end of week	No. of surviving negative controls at end of week
0[1]	0[2]	-	-
1	0	depletion diet (72)[3]	8
2	0	1000 ANRC[4] (72)	8
3	0	1000 ANRC (72)	8
4	0	1000 ANRC (72)	8
5	0	1000 ANRC (72)	7
9 chicks sacrificed[5] at the end of the fifth week			
6	I	depletion diet (11)	6
	II	1000 alfalfa[6] (11)	
	III	2000 alfalfa (11)	
	IV	500 alfalfa & 500 ANRC (10)	
	V	1000 alfalfa & 1000 ANRC (10)	
	VI	1000 ANRC (10)	
5 chicks from groups I and V, and 4 from groups II, III, IV, and VI sacrificed[7] at the end of the sixth week			
7	I	depletion diet (6)	5[8]
	II	1000 alfalfa (7)	
	III	2000 alfalfa (7)	
	IV	500 alfalfa & 500 ANRC (6)	
	V	1000 alfalfa & 1000 ANRC (5)	
	VI	1000 ANRC (6)	
5 chicks from group IV, and 4 from each other group sacrificed[7] at the end of the seventh week			

[1]Start of experiment; 80 newly hatched chicks, 8 were used for negative controls.

[2]All chicks received same treatment.

[3]Number of chicks still living in parentheses.

[4]Units of vitamin A potency per kg of feed supplied by ANRC vitamin A standard.

[5]Chicks were sacrificed for vitamin A assay of the sera and livers; remaining chicks randomized (10 or 11/group) into groups I - VI.

[6]Units of vitamin A potency per kg of feed supplied by alfalfa meal.

[7]Chicks were sacrificed for vitamin assay of the sera and livers.

[8]Chicks in advanced stage of vitamin A deficiency.

TABLE 5

Outline of experiment IV

Experimental period (week)	Group number	Type of diet and number of chicks at end of week	No. of surviving negative controls at end of week
0[1]	0[2]	-	-
1	0	depletion diet (74)[3]	8
2	A	1000 alfalfa[4] (37)	8
	B	1000 ANRC[5] (37)	
3	A	1000 alfalfa (37)	8
	B	1000 ANRC (37)	
4	A	1000 alfalfa (37)	7
	B	1000 ANRC (37)	
5	A	1000 alfalfa (37)	7
	B	1000 ANRC (37)	
4 chicks from group A and 5 from group B sacrificed[6] at the end of the fifth week			
6	AI	depletion diet (11)	6
	AII	1000 alfalfa (11)	
	AIII	1000 ANRC (12)	
	BI	1000 alfalfa (11)	
	BII	1500 alfalfa (10)	
	BIII	1000 ANRC (10)	
4 chicks from each group sacrificed[7] at the end of the sixth week			
7	AI	depletion diet (7)	5[8]
	AII	1000 alfalfa (7)	
	AIII	1000 ANRC (8)	
	BI	1000 alfalfa (7)	
	BII	1500 alfalfa (6)	
	BIII	1000 ANRC (6)	
4 chicks from each group sacrificed[7] at the end of the seventh week			

[1]Start of experiment; 82 newly hatched chicks, 8 were used for negative controls.

[2]All chicks received same treatment; chicks randomized (37/group) at end of depletion period.

[3]Number of chicks still living in parentheses.

[4]Units of vitamin A potency per kg of feed supplied by alfalfa meal.

[5]Units of vit. A potency per kg of feed supplied by ANRC vit. A standard.

[6]Chicks were sacrificed for vit. A assay of sera and livers; remaining chicks from group A randomized (11 or 12/group) into groups AI, AII and AIII, and from group B randomized (10 or 11/group) into groups BI, BII and BIII.

[7]Chicks were sacrificed for vitamin A assay of the sera and livers.

[8]Chicks in advanced stage of vitamin A deficiency.

Determination of Vitamin A Activity of Alfalfa

Extraction of Beta-Carotene from Alfalfa. The AOAC method (33) was followed for the determination of vitamin A activity of alfalfa. The reagents used were dry acetone[5] and commercial hexane.[6] Acetone-hexane mixture was prepared by mixing 30 ml acetone with 70 ml hexane.

Three samples of 1.000 g dehydrated alfalfa meal[4] were weighed, and each placed in a 125 ml boiling flask. Thirty milliliters of the acetone-hexane mixture was added to each flask and the material refluxed[7] for an hour at a rate of 1 to 3 drops per second. At the end of that period, the condenser drip tip was rinsed with hexane from a pipette. After cooling the sample to room temperature, the extract was filtered through cotton in a funnel into a 100 ml volumetric flask and the residue washed with hexane. The filtrate was made to volume with hexane. The final solution contained 9% acetone.

Separation of Pigments. A chromatographic column was used for separation of the pigments. The adsorbent was a mixture of 50 g magnesium oxide[8] and 50 g diatomaceous earth.[9]

To prepare the column, a cotton plug was placed inside a glass tube of 11 to 12 mm inside diameter, joined to another 5 mm tube. Loose adsorbent

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[5]ACS reagent grade, free of alcohol.

[6]Skellysolve B, Skelly Oil Co., redistilled, collecting fraction boiling at 64-68° C.

[7]Ground glass connections on flask and condensor, and on all stoppered equipment.

[8]MgO, Seasorb 43, Westvaco Chemical Co.

[9]Supercel, Johns Manville Co.

was added to 15 cm depth, and the tube attached to a suction flask, applying full vacuum from a water pump. An inverted cork mounted on a rod was used to tap the column and to gently press the adsorbent and flatten the surface. The packed column was about 10 cm deep. A 1 cm layer of sodium sulfate[10] was placed above the adsorbent.

With the vacuum continuously applied, the carotenoid extract from alfalfa was poured onto the column and allowed to absorb. Just as all was absorbed, about 50 ml 10% acetone in hexane was added to develop the chromatogram and wash carotene through the adsorbent. During the entire operation the top of the column was kept covered with developer solvent.

After the chromatogram was developed, the colored bands of xanthophylls, chlorophylls, carotene and their oxidation products were observed on the column. The yellow eluate of the lowest diffused band was collected in a 100 ml volumetric flask and made up to volume with hexane.

Colorimetric Procedure. Carotene content was determined by measuring the intensity of light through a 440 mμ filter in an Evelyn photoelectric colorimeter (34). The instrument had been calibrated with solutions of pure beta-carotene, and the factor 2.86, obtained from the calibration curve, was used to convert absorbance to carotene concentration per ml solution.

The solution of carotene absorbs according to Beer's Law. Galvanometer readings, designated as T values, were converted to density values, L, as follows:

$$L = \log \frac{T_0}{T_s} = \log \frac{100}{T_s} = 2 - \log T_s$$

where T_0 = galvanometer reading at zero concentration

[10]ACS reagent grade, powdered, anhydrous.

T_s = galvanometer reading at sample concentration.

$$\mu g \text{ beta-carotene} = L_{440} \times \frac{V}{G} \times \text{factor.}$$

where V = volume containing extract from sample.

G = sample weight

factor = 2.86

Sample Calculation: Vitamin A Activity in Alfalfa. An alfalfa sample was assayed following the procedure just described.

$$T_{440} = 56.75$$

thus, $L_{440} = 0.2464$.

$$0.2464 \times \frac{200}{1} \times 2.86 = 141 \mu g \text{ carotene per g alfalfa.}$$

One μ gram carotene is equivalent to 1.67 IU vitamin A.

Thus, $141 \times 1.67 = 235$ IU vitamin A per g alfalfa.

Determination of Carotene and Vitamin A in Serum

Carotene Analysis. A modified Kimble method (35) was used. Reagents were ethanol[11] and petroleum ether.[12] Sera were obtained as mentioned earlier.

Into glass stoppered centrifuge tubes[7] were pipetted 5 ml serum, 5 ml ethanol, and 13 ml petroleum ether. The tubes were stoppered tightly, sealing with starch-glycerin lubricant, and shaken for 10 minutes on a mechanical shaker. Tubes were centrifuged at low speed for 2 minutes, and the supernatant, containing petroleum ether extract, was carefully drawn off through a suction device into a test tube. A 10 ml aliquot of the extract was pipetted to an Evelyn tube.[13] Colorimetric analysis of the beta-carotene was similar to the procedure outlined for the determination of beta-carotene from alfalfa.

$$\text{Carotenoids } (\mu g \text{ per } 100 \text{ ml}) = L_{440} \times 2.86 \times 13 \times \frac{100}{\text{volume serum}}$$

where 2.86 = calibration factor.

Vitamin A Analysis. The method, a modified Kimble procedure (35), was based on the Carr-Price reaction, wherein 20% antimony trichloride solution was used as reagent. The reagent was prepared by dissolving 20 g pure white antimony trichloride crystals[14] in chloroform[15] under moderate heating,

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[11] 95% ethanol, ACS reagent grade, free of aldehyde when tested by Schiff's reaction.

[12] Skellysolve F, Skelly Oil Co., redistilled, collecting portion boiling at 37-42° C.

[13] 7 × 7/8 inch soft glass test tube, calibrated for uniformity.

[14] ACS reagent grade, Mallinckrodt Chemical Works: reagent usually made in batches of 1000 or 2000 ml.

[15] ACS reagent grade.

making to 100 ml with chloroform, allowing to settle, and filtering rapidly. Three milliliters fresh acetic anhydride[15] was added per 100 ml and the reagent was stored in a brown glass-stoppered bottle.

The 10 ml petroleum ether extract previously used for the carotene determination was evaporated to dryness in the Evelyn tube under vacuum, using a water bath at 60-65° C for heating. One milliliter chloroform was added as the vacuum was broken to completely dissolve the residue. This preparation was used for the colorimetric determination in the Evelyn photoelectric colorimeter with a 620 mμ filter.

Nine milliliters of the Carr-Price reagent was added from a rapid delivery automatic dispenser. The galvanometer reading was taken at the point of temporary stability occurring within less than 5 seconds after delivery of the reagent. In the reaction a deep blue color is developed.

Since carotenoids also produce a blue color with antimony trichloride (36, 37), a correction is made in the calculation of vitamin A. By calibration with carotene solution it was found that 0.14 L_{440} subtracted from L_{620} gave the corrected L_{620} value for vitamin A. The calibration factor relating weight of vitamin A to the corresponding L value was 13.1, hence μg vitamin A in the sample in 1 ml chloroform was $L_{620} \times 13.1$.

Sample Calculation: Vitamin A in Serum.

Let $T_{440} = 87.75$ and $T_{620} = 86.25$,

Thus $L_{440} = 0.0667$ and $L_{620} = 0.0642$.

Concentration of carotenoids:

$$L_{440} \times \text{factor} \times \text{vol. petroleum ether} \times \frac{100}{\text{volume serum}}$$

= μg carotenoids per 100 ml serum.

Thus, $0.0667 \times 2.86 \times 13 \times \frac{100}{5} = 49.6$ μg carotenoids per 100 ml serum.

Calculation of Vitamin A Concentration.

corrected L_{620} = L_{620} - 0.14 L_{440} = 0.0642 - 0.0093 = 0.0549.

$$\text{corrected } L_{620} \times \text{factor} \times \frac{\text{vol. petroleum ether}}{\text{volume sample}} \times \frac{100}{\text{volume serum}} = \text{conc.vit.A}$$

Thus, $0.0549 \times 13.1 \times \frac{13}{10} \times \frac{100}{5} = 18.7$ μg vitamin A per 100 ml serum,

or, converted to standard units,

18.7 × 3.34 units = 61.8 IU per 100 ml serum.

Determination of Vitamin A in Liver

Saponification, or alkaline hydrolysis, was used to release fat soluble vitamin A from liver tissue, converting vitamin A esters to their alcohols; lipids and the peptide chains are hydrolyzed.

Saponification. Reagents used were 50% KOH[15] and 95% ethanol.[11] Ten grams of each liver was used when the total weight was more than 10 g. To get representative samples, large livers were cut into pieces of about 1 g, and the proper quantity of tissue taken at random. Liver was weighed in a 125 ml boiling flask,[7] using a Chainomatic analytical balance.

To each sample was added 15 ml of 95% ethanol, and 10 ml of 50% KOH. Flasks were attached to condensers[7] and samples were refluxed by gentle boiling on a steam bath or hot plate for 30 minutes. Bumping of solution, especially when using a hot plate, was prevented by placing a few glass beads in each flask. Material in the flask was shaken twice during the process to help complete the digestion.

Extraction of Vitamin A. Reagents were 95% ethanol,[11] ether,[16] distilled water, and sodium sulfate.[10]

Flasks containing the digested material were cooled immediately to room temperature under running water. The contents were transferred to 250 ml separatory funnels, rinsing flasks first with 20 ml distilled water, then, 5 ml ethanol, and finally with the 50 ml ether used for extraction. Extraction was carried out by shaking vigorously for 2 minutes. The layers were allowed to separate, after which the lower layer in each funnel was drained to a second separatory funnel containing 40 ml ether, and shaken for 2 minutes. After layers separated, the lower layer was discarded from the second separatory funnel. If emulsions were formed that did not separate in a reasonable time, 1 to 2 ml ethanol was added, followed by swirling of the solution and allowing it to stand until phases separated.

Extracts were washed to remove soaps, alkali, alcohol, etc., by mixing gently the extract in the first separatory funnel with 100 ml distilled water, and after separation, draining the lower layer to the second separatory funnel and repeating the washing. The washing to remove alkali in each funnel in turn, as described, was repeated 2 times by shaking the extracts with 50 ml of alcoholic wash.[17] The ether extracts in the 2 separatory funnels were combined in one and 10 ml hexane[6] added to reduce the solubility of water in ether. The solution was washed by shaking a final time with 50 ml distilled water. The water was drained after layers separated. Five to eight grams anhydrous sodium sulfate was added to the

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[16] Anhydrous, ACS reagent grade, free of peroxides when tested with Jorrison's reagent (Baskerville, C., and W. A. Hamor, 1911, Ind. and Engr. Chem., 3: 378-398).

[17] Mixture of 10% ethanol and 0.1% HCl in distilled water.

separatory funnel and shaken to remove any water disolved in the ether. The extract was poured carefully from the top of the separatory funnel through a funnel containing a small cotton plug filter and some anhydrous sodium sulfate into a 100 ml volumetric flask. The sodium sulfate was washed 3 times by shaking with small volumes of ether. The solution made to volume in the flask with dry ether, and mixed thoroughly.

From this point the procedure was similar to the determination of vitamin A from serum extracts.

Sample Calculation: Vitamin A in Liver. In terms of standard units of vitamin A, 43.3 was the factor that had been determined for the instrument used to relate vitamin A potency to the corresponding L value. Corrected L value was obtained as explained for the serum vitamin A determination.

For a 10 g sample of liver $T_{440} = 96.25$ and $T_{620} = 91.25$,

thus, $L_{440} = 0.0166$ and $L_{620} = 0.0386$.

Corrected $L_{620} = L_{620} - 0.14\ L_{440} = 0.0386 - 0.0023 = 0.0363$

$$\text{Corrected } L_{620} \times 43.3 \frac{1}{\text{sample weight}} \times \frac{\text{volume ether}}{\text{volume used}} = \text{units vit. A per g liver}$$

$$\text{Thus, } 0.0363 \times 43.3 \times \frac{1}{10} \times \frac{100}{10} = 1.6 \text{ IU vitamin A per g liver.}$$

RESULTS AND DISCUSSION

Data from the 4 experiments are presented in tables 6, 7, 8, and 9,[18] and are results of serum and liver assays at the end of the pretest period (1 week depletion, and 4 weeks of feeding of either 1000 units of vitamin A or 1000 units vitamin A activity from alfalfa per kg feed) and after a switch to the experimental diet for 1 or 2 weeks. Experiment I was carried out as a preliminary trial and was terminated after the chicks were given the experimental diet for 1 week only. The results of this trial, as shown in table 6, were an indication that serum and liver vitamin A levels dropped considerably after beta-carotene from alfalfa was substituted for the equivalent quantity of pure vitamin A. Lower levels of serum and liver vitamin A also were observed in groups where 2000 or 3000 vitamin A units from alfalfa was substituted for 1000 units pure vitamin A. This indicated a decreased utilization of beta-carotene which might have been due to a conditioning effect of pure vitamin A fed in the pretest period. This indication was supported by weight gain and feed conversion ratio data, which showed a lesser efficiency of alfalfa to promote growth as compared to pure vitamin A.

In experiments II and III, basically the same trials were repeated. For additional information combinations of alfalfa and ANRC vitamin A mixtures were used as supplements to the test diet. Results of experiment II (table 7) were similar to those of experiment III (table 8). Vitamin A levels of serum and liver dropped after beta-carotene was substituted for the equivalent potency of vitamin A. When beta-carotene was supplemented

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[18] See appendix, tables AI - AIV, for individual analytical results.

TABLE 6

Results of Serum and Liver Vitamin A Assays, Experiment I

Group number	Type of diet	Vitamin A level in serum units/100 ml	Vitamin A level in liver units/liver	Weight gain[1] (or loss) g/chick	Feed conversion ratio[1,2]
0	1000 ANRC[3]	75.9	8.8	366	2.3
I	depletion[4]	15.7	4.7	-53	-
II	1000 alfalfa	20.3	4.5	62	3.7
III	2000 alfalfa	20.7	6.3	59	3.2
IV	3000 alfalfa	42.4	7.3	77	3.3
V	4000 alfalfa	55.5	7.9	74	3.2
VI	1000 ANRC	66.8	11.7	109	2.5

[1]Determined for chicks not sacrificed or dying over a 4-week prefeeding period and a 1-week test period.

[2]Ratio of amount of feed consumed to increase in body weight.

[3]Four weeks pretest diet; see experimental design table 2.

[4]One week test diet; see experimental design table 2.

TABLE 7

Results of Serum and Liver Vitamin A Assays, Experiment II

Group number	Type of diet	Vitamin A level in serum units/100 ml		Vitamin A level in liver units/liver		Weight gain[1] g/chick	Feed conversion ratio[1,2]
0	1000 ANRC[3]	77.4		17.9		263	2.0
		week[4] 1	2	week[4] 1	2		
I	depletion	13.5	11.2	12.6	5.3	179	1.6
II	1000 alfalfa	59.1	69.8	12.5	20.2	290	2.6
III	2000 alfalfa	84.2	105.4	15.8	37.6	259	2.6
IV	500 alfalfa & 500 ANRC	74.8	78.4	12.7	11.7	281	2.8
V	1000 alfalfa & 1000 ANRC	154.1	115.0	24.0	46.2	259	2.7
VI	4000 alfalfa	206.8	220.8	73.3	131.7	272	2.6
VII	1000 ANRC	97.5	102.6	19.9	13.5	237	2.6

[1]Determined for chicks not sacrificed or dying over a 4-week prefeeding period and a 2-week test period.

[2]Ratio of amount of feed consumed to increase in body weight.

[3]Four weeks pretest diet; see experimental design table 3.

[4]After 1 week on test diet chicks not sacrificed or dying continued on same diet for an additional week.

TABLE 8

Results of Serum and Liver Vitamin A Assays, Experiment III

Group number	Type of diet	Vitamin A level in serum units/100 ml		Vitamin A level in liver units/liver		Weight gain[1] g/chick	Feed conversion ratio[1,2]
0	1000 ANRC[3]	66.8		13.7		353	2.6
		week[4] 1	2	week[4] 1	2		
I	depletion	12.2	16.5	2.7	12.5	210	3.6
II	1000 alfalfa	36.0	35.7	7.1	14.6	199	3.4
III	2000 alfalfa	74.7	106.8	26.1	31.2	185	3.2
IV	500 alfalfa & 500 ANRC	64.2	75.0	13.6	14.0	235	3.4
V	1000 alfalfa & 1000 ANRC	111.5	112.8	30.2	29.3	182	3.1
VI	1000 ANRC	72.1	87.4	27.2	35.1	235	3.1

[1]Determined for chicks not sacrificed or dying over a 4-week prefeeding period and a 2-week test period.

[2]Ratio of amount of feed consumed to increase in body weight.

[3]Four weeks pretest diet; see experimental design table 4.

[4]After 1 week on test diet chicks not sacrificed or dying continued on same diet for an additional week.

TABLE 9

Results of Liver and Serum Vitamin A Assays, Experiment IV

Group number	Type of diet	Vitamin A level in serum units/100 ml		Vitamin A level in liver units/liver		Weight gain[1] g/chick	Feed conversion ratio[1,2]
A	1000 alfalfa[3]	61.7		7.2		355	2.5
B	1000 ANRC[3]	77.8		11.5		360	2.4
		week[4] 1	2	week[4] 1	2		
AI	(1000 alfalfa)[5] depletion	19.3	11.4	4.8	2.8	214	3.5
AII	(1000 alfalfa) 1000 alfalfa	78.9	59.3	9.7	10.5	247	3.4
AIII	(1000 alfalfa) 1000 ANRC	94.2	67.2	13.5	18.5	263	3.1
BI	(1000 ANRC) 1000 alfalfa	73.6	59.8	17.4	16.2	276	2.9
BII	(1000 ANRC) 1500 alfalfa	88.4	72.5	19.7	33.1	289	2.9
BIII	(1000 ANRC) 1000 ANRC	127.8	78.3	23.5	22.4	262	3.0

[1]Determined for chicks not sacrificed or dying over a 4-week prefeeding period and a 2-week test period.

[2]Ratio of amount of feed consumed to increase in body weight.

[3]Four weeks pretest diet; see experimental design table 5.

[4]After 1 week on test diet chicks not sacrificed or dying continued on same diet for an additional week.

[5]Pretest diet in parentheses.

in the test diet at a potency twice as high as pure vitamin A, serum and liver vitamin A levels were in the same range, except for liver data at the end of the second week of experiment II. These data from experiments II and III, with the exception of liver data at the end of the second week of group VI experiment II, suggested a conditioning effect. In contrast to results of experiment I growth data and feed conversion ratios indicated equal or even greater gains when equivalent vitamin A activities from alfalfa and pure vitamin A were placed in the test diet. Weight gain of group II experiment III was, however, an exception to this.

Based on equivalent vitamin A potencies, the mixtures of alfalfa and vitamin A were more effective than alfalfa and less effective than pure vitamin A when serum and liver vitamin A were taken as parameter. The mixture of 1000 IU ANRC vitamin A standard and 1000 IU vitamin A from alfalfa is of interest because even in the presence of ANRC vitamin A standard, vitamin A activity of alfalfa is utilized.

As Castano et al. (38) postulated, chicks seemed to utilize vitamin A better than carotene. This was also in agreement with the findings of Rousseau et al. (39) on Holstein calves. They employed blood and liver concentrations of vitamin A to determine the relative value of carotene from dehydrated alfalfa, and of vitamin A from a dry carrier. Based on these criteria they came to the conclusion that the calves did not utilize carotene as efficiently as equal units of vitamin A, given as a mixture of naturally occurring vitamin A esters.

The relative vitamin A potencies of vitamin A and carotene were not the objective of this work, which is to find out whether chicks, after being conditioned to the use of pure vitamin A, will less efficiently utilize subsequently fed equivalent quantity of vitamin A or beta-carotene

from alfalfa source. Hence, two factors need to be considered regarding the possibility of inefficient use of beta-carotene. In the first place, the possibility exists that the two vitamin A sources supplying equivalent potencies of vitamin A are not utilized to the same degree by the chick. A second factor, conditioning, has to be considered. This might put additional stress on utilization of beta-carotene as compared to pure vitamin A.

In experiment IV beta-carotene from alfalfa was fed in the pretest diet of a group of chicks. This was done to compare the effect of type of pretest diet upon the utilization of beta-carotene in the test diet. As indicated in table 5, the chicks were divided into 2 groups for the pretest diet. One group received alfalfa and the other vitamin A standard, supplemented in the diet at a potency of 1000 IU per kg of feed. Results from serum and liver vitamin A assays at the end of a 4-week feeding period (table 9) showed that the chicks fed the alfalfa meal already had lower levels of serum and liver vitamin A than the chicks fed the pure vitamin A, before the switch of diet. Hence, one of the reasons lower values were obtained from group AIII chicks compared to group BIII chicks in experiment IV, could be a lower serum and liver supply of vitamin A before receiving the experimental diet.

It is not possible to judge from the data presented in table 9 whether there was adaptation to the use of pure vitamin A, which resulted in decreased values for group BI as compared to group BIII. When serum vitamin A levels of group AII were compared with group BI, one could make a case for adaptation since group B chicks had higher level of serum vitamin A than group A chicks at the end of the prefeeding period. Group BI chicks

possibly were conditioned to the use of pure vitamin A, and hence could not metabolize beta-carotene from the alfalfa source with the same degree of efficiency as group AII chicks which remained on alfalfa diet after depletion period.

However, the higher liver values of group BI chicks, as compared to group AII, makes interpretation more complicated. In contrast to previous experiments in this work, where beta-carotene was substituted for equivalent potency of vitamin A, chicks showed higher values of liver vitamin A after 1 week on test diet than before the switch of diet. In fact, both the serum and liver values were relatively higher than would be expected based on the previous experiments. It is possible that genetic, environmental or even biochemical individuality factors were involved.

Because of inconsistency of data, it is not possible to draw definite conclusions on whether there is a conditioning effect when pure vitamin A is replaced by an equivalent quantity of beta-carotene in the chicks diet. Further investigations are needed, taking the following factors into special consideration:

a) serum and liver vitamin A levels when chicks are fed alfalfa starting right after the depletion period through the entire experiment.

b) serum and liver vitamin A levels when chicks receive alfalfa as source of vitamin A potency subsequent to receiving a diet with pure vitamin A supplementation.

Values from (a) will serve as reference data to check whether results obtained from (b) are caused by the basically different way of metabolizing beta-carotene and vitamin A (40, 41), or from being conditioned previously to the use of pure vitamin A. If adaptation effects can be traced, feeding

of the trial diet for various periods of time is suggested to observe whether a certain latent period can be detected after the switch of diet. This is the period in which the systems of the body are undergoing biological adjustment to the new nutrient. In most cases of adaptation the organism resumes normal metabolism after adjustment for a certain length of time; this then marks the end of the latent period.

Biological variation is a frequent problem encountered when working with experimental animals. Williams (42) pointed out that since genes are so diverse one should expect that the biochemical reactions taking place in any specific organ or tissue would vary in efficiency from individual to individual.

Since interpretation of the data in this work is based on relative values, each result is compared to other results of the same experiments. The result that was obtained more or less consistently in all 4 experiments was the vitamin A level per 100 ml of serum and per gram of liver tissue (see appendix) at the end of the pretest period of 1000 ANRC vitamin A per kg of feed. This is a reference point for results of the test diets, but because of variation among results of individual experiments, definite conclusions are not possible on the effect of test nutrients following ingestion of a previously fed nutrient.

Data expressing the feed conversion ratio, i.e. the ratio of the quantity of feed consumed and the growth of the chick, did not give any additional information on conditioning. Feed conversion ratios determined over 4 weeks of prefeeding were smaller than those when test diets were fed (one week in experiment I, and two weeks in experiment II, III and IV). Weight data at the end of the experiment (see appendix) indicated similar

potencies of equivalent units of beta-carotene and vitamin A to promote growth, as shown by Parrish et al. (43).

The deficiency of the basal diet was tested by placing a group of birds on negative control in each experiment. These birds received the vitamin A deficient basal diet from the start of the experiment and were observed for symptoms due to lack of vitamin A. The period before the first birds in each control group started to show deficiencies varied between 10 and 23 days. A ruffled appearance, weak legs and dull eyes with exudate in the advanced stages were the usual symptoms observed on the depleted (negative control) birds. Most negative controls did not survive very long after these symptoms appeared. In post mortem investigations the most striking abnormality was the accumulation of solid, white ureate in the enlarged ureters. Pustules along the esophagus and the trachea were only clearly visible in a few chicks, usually those that survived the depletion for the longer times. None of the negative controls survived experiments I and II. There were 5 negative control chicks alive, though in poor condition, at the end of experiments III and IV.

For further investigation, studies could be performed on the mode of intestinal absorption of beta-carotene and pure vitamin A. It was demonstrated by Cheng and Deuel (44) that the wall of the small intestine of the chick was the site of conversion of orally administered beta-carotene to vitamin A. In addition, Sibbald and Hutcheson (45) observed that the conversion of beta-carotene to vitamin A in a ligatured duodenal loop of a living chick took place in the duodenal wall. Shellenberger (46) concluded that carotene absorption was one possible factor limiting the conversion of carotene to vitamin A. Based on these findings it would be worthwhile to

look for the effects of prefeeding conditioning in conjunction with intestinal absorption. One possibility would be an experiment similar to Munck's (47). He demonstrated that preloading of rat intestine with one amino acid could establish the conditions necessary for competitive inhibition of the transport of other amino acids.

Another possibility of a detailed study in nutrient adaptation is to study changes in enzyme activities. Freedland and Harper (48, 49), and Fitch and Chaikoff (50) have shown that changes in the type of dietary carbohydrate induced adaptations in the activities of some of the glycolytic enzyme systems in the rat. An experiment carried out by Carroll (4) is another example of this type of study.

The field of nutrient conditioning is still open to many investigations. Fisher et al. (32) have demonstrated that utilization of a nutrient could be affected by prefeeding of another nutrient. Carroll's experiment (4) was an indication that altered response of the animal due to nutrient conditioning could be the result of modified enzyme activity. Experiments conducted at Mississippi State University (51) showed that when chicks were previously fed high levels of vitamin A, the apparent absorption of carotenoids was decreased. The effect of prefeeding as studied in this work is just one among many possible examples of studies of nutrient conditioning.

SUMMARY

Four experiments were conducted to determine whether chicks could be conditioned to the use of pure vitamin A at the expense of other vitamin A sources. Observed was the capacity of chicks to utilize beta-carotene from an alfalfa meal after they had been fed pure vitamin A for a certain length of time. Performance of the chicks was judged on the basis of serum and liver vitamin A levels.

Newly hatched chicks were depleted of their initial vitamin A reserves for a 1-week period. They then were fed a diet (pretest diet) for 4 weeks which was supplemented with 1000 units vitamin A activity per kg of feed from either ANRC vitamin A standard or alfalfa meal. Following the pretest diet, chicks were randomized into several groups. Each group received a test diet with graded vitamin A activity. Sources of vitamin A were ANRC vitamin A standard, or alfalfa meal, or a mixture of both. Test diets were fed for 1-week or 2-week periods. At intervals several chicks were sacrificed and the sera and livers were assayed for vitamin A.

Values of serum and liver vitamin A before and after a switch of diet were compared. The relative changes were used as an indication of a nutrient conditioning effect. Results were not consistent but they showed a general decreased capacity of the chick to utilize beta-carotene from alfalfa after they had been previously fed pure vitamin A. This indicated that chicks might be conditioned to the use of pure vitamin A. Weight gains and feed conversion ratio data did not contribute additional information regarding the conditioning effect of previously fed pure vitamin A.

Since results were not entirely consistent, and the problem of nutrient conditioning has many aspects that have not been studied, more investigations in this field should be conducted.

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APPENDIX

TABLE A1

Results of Serum and Liver Analysis
of Experiment I

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids µg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids µg/g liver	Vit A units/ g liver	Vit A units/ liver
2248	0	1000	4	437	7.9	40.3	83.5	1.2	1.0	7.7
2243		ANRC		492	11.1			1.1	1.1	10.7
2261	0	1000	4	399	9.8	46.51	76.28	1.0	0.7	6.8
2221		ANRC		464	9.1			0.8	1.0	8.7
2236	0	1000	4	451	8.6	43.0	82.9	1.4	1.2	10.7
2272		ANRC		421	8.6			1.0	1.4	11.8
2266				360	- *			-	-	-
2255	0	1000	4	415	9.8	37.7	61.1	0.9	0.7	6.4
2205		ANRC		439	9.7			0.8	0.8	8.0
2258				444	- *			-	-	-
2210	I	basal	1	360	7.3	15.8	12.2	0.9	0.9	6.3
2237				378	8.2			0.9	0.6	5.2
2267	I	basal	1	386	10.4	11.0	18.7	0.7	0.2	2.2
2229				364	6.0			1.5	0.9	5.3
2216	II	1000	1	412	10.0	22.4	20.1	0.8	0.5	4.5
2208		alf.		452	10.8			0.7	0.4	4.1
2202	II	1000	1	417	14.7	22.4	21.5	0.8	0.3	4.1
2246		alf.		542	12.8			0.8	0.4	5.2
2238	III	2000	1	550	10.4	20.7	20.5	0.7	0.4	4.6
2213		alf.		453	13.9			0.9	1.2	16.4
2204	III	2000	1	530	12.7	19.1	20.8	0.7	0.4	5.3
2254		alf.		566	15.2			0.6	0.6	9.0
2222	IV	3000	1	464	13.0	42.1	42.2	1.1	1.0	12.0
2265		alf.		516	11.1			0.9	0.5	5.2

TABLE A1 (Continued)

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids μg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids μg/g liver	Vit A units/ g liver	Vit A units/ liver
2273	IV	3000	1	535	13.6	40.3	42.5	0.9	0.4	5.2
2241		alf.		564	11.3			1.1	0.6	6.6
2217	V	4000	1	519	13.2	47.4	61.7	-**	-	-
2262		alf.		486	12.2			1.3	0.5	5.7
2231	V	4000	1	517	13.8	47.4	49.3	1.0	0.7	9.9
2242		alf.		447	8.8			1.1	1.0	8.2
2234	VI	1000	1	513	13.4	15.8	63.2	0.7	1.5	19.6
2211		ANRC		571	13.3			0.6	0.9	12.4
2212	VI	1000	1	568	12.0	20.7	70.4	1.0	0.5	6.2
2259		ANRC		522	10.2			1.0	0.8	8.4

[1]Four weeks pretest diet followed by one week test diet

[2]Serum samples obtained from pooled blood of 2 or 3 chicks as grouped in table.

*Liver not saved for analysis.

**Sample lost during analysis.

TABLE A2

Results of Serum and Liver Analysis
of Experiment II

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids µg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids µg/g liver	Vit A units/ g liver	Vit A units/ liver
1987	0	1000	4	446	11.5	38.6	70.8	0.9	1.2	13.2
1917		ANRC		543	12.4			0.8	1.7	21.0
1913				475	9.9			0.6	1.6	14.9
1947	0	1000	4	447	9.5	38.6	73.7	1.0	1.6	14.8
2099		ANRC		465	10.5			1.0	1.7	16.7
2006				527	12.3			0.8	2.5	31.0
2025	0	1000	4	428	10.0	39.4	91.0	0.9	1.4	13.7
1977		ANRC		450	8.9			-*	-	-
2016				437	11.9			-*	-	-
1935				504	9.9			-*	-	-
1918	I	basal	1	653	14.0	42.1	13.2	0.9	0.6	8.0
1936				583	12.0			0.8	0.4	4.5
2022	I	basal		463	10.9	40.4	13.7	0.8	1.3	13.8
2013				579	12.9			0.8	1.9	24.2
2003	II	1000	1	558	10.9	65.9	53.5	1.1	1.4	14.9
2001		alf.		575	12.7			1.1	0.9	10.8
1944	II	1000	1	500	11.7	66.9	64.6	1.0	1.0	11.9
2095		alf.		670	15.9			0.9	0.8	12.2
2096	III	2000	1	600	13.9	86.7	89.7	0.9	1.5	23.6
1972		alf.		636	16.6			0.9	1.1	17.4
2091	III	1000	1	616	-*	83.6	78.9	-	-	-
1916		alf.		585	14.0			0.8	0.9	12.7
2089				586	13.0			1.1	0.7	9.3

TABLE A2 (Continued)

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids µg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids µg/g liver	Vit A units/ g liver	Vit A units/ liver
1902	IV	500 alf. & 500	1	516	12.6	53.8	60.3	0.9	0.7	8.3
1986				534	13.5			0.9	1.5	19.3
1942	IV	ANRC	1	548	13.4	56.5	89.3	0.7	0.9	11.9
2009				563	11.4			1.2	1.0	11.4
1908				479	-*			-	-	-
2010	V	1000 alf. & 1000	1	534	12.1	79.6	156.6	1.1	1.6	18.3
2012				545	13.0			1.0	2.6	32.7
1979	V	ANRC	1	587	12.4	70.7	151.5	0.9	1.5	17.8
1976				529	12.1			0.9	2.3	27.1
1927				602	-*			-	-	-
2017	VI	4000 alf.	1	514	13.2	115.8	188.5	1.5	4.8	61.5
2015				582	13.5			2.4	8.7	114.4
1928	VI	4000 alf.	1	518	13.5	184.3	225.0	1.5	2.3	30.0
1991				553	14.0			2.6	6.4	87.1
1961	VII	1000 ANRC	1	552	10.4	39.4	95.7	1.0	1.9	18.9
2018				538	12.3			0.8	2.1	25.0
2021	VII	1000 ANRC	1	609	12.4	37.3	99.2	0.6	1.2	14.9
2024				603	13.6			0.5	1.6	20.9
2026	I	basal	2	641	10.5	50.1	15.3	1.0	0.6	5.8
2090				663	13.0			0.5	0.5	6.7
1973	I	basal	2	533	15.6	37.7	7.8	0.4	1.7	26.3
2004				613	10.9			0.7	0.6	6.4
1919	II	1000 alf.	2	793	15.4	71.7	76.7	0.9	1.0	15.6
1995				790	19.3			0.8	1.4	25.2
2002	II	1000 alf.	2	642	10.7	61.2	62.8	0.8	1.2	12.6
1983				716	16.5			0.8	1.4	22.9

TABLE A2 (Continued)

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids μg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids μg/g liver	Vit A units/ g liver	Vit A units/ liver
1965	III	2000	2	752	16.1	99.2	105.5	1.0	1.8	28.4
1953		alf.		709	13.8			1.4	3.9	53.7
1960	III	2000	2	671	14.6	79.6	105.2	1.4	2.2	31.5
2093		alf.		680	13.8			1.2	2.1	29.2
1993	IV	500	2	717	14.0	51.9	78.1	0.7	0.8	11.4
1996		alf. & 500		762	15.0			0.9	1.1	15.9
1914	IV	ANRC	2	812	14.3	49.2	78.6	0.8	0.7	10.1
1933				697	11.9			0.8	0.8	9.5
1983	V	1000	2	674	13.6	49.2	116.8	0.6	1.6	21.5
2008		alf. & 1000		752	14.9			1.0	4.8	71.7
1963	V	ANRC	2	717	12.7	44.7	113.2	0.9	4.1	51.0
1966				696	14.8			0.9	2.8	40.7
1949	VI	4000	2	674	12.9	181.6	267.0	2.4	9.8	125.5
1958		alf.		688	13.1			2.2	17.3	224.5
1943	VI	4000	2	719	12.8	144.9	174.5	1.9	7.6	96.2
1946		alf.		623	12.7			1.8	6.4	80.4
1984	VII	1000	2	623	12.3	33.4	126.5	0.8	1.1	13.5
2023		ANRC		707	13.8			0.8	0.7	9.7
2094	VII	1000	2	658	13.1	35.9	78.6	0.6	1.6	20.3
2014		ANRC		710	14.1			0.5	0.8	10.6

[1]Four weeks pretest diet followed by 1 week and 2 weeks test diet.

[2]Serum samples obtained from pooled blood of 2 or more chicks as grouped in table.

*Liver not analyzed.

TABLE A3

Results of Serum and Liver Analysis
of Experiment III

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids µg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids µg/g liver	Vit A units/ g liver	Vit A units/ liver
4647	0	1000	4	448	8.9	43.8	72.5	0.8	0.9	8.2
4617		ANRC		424	9.3			1.1	1.0	9.6
4672				464	10.3			0.7	3.0	31.1
4667	0	1000	4	425	8.9	43.0	61.4	1.4	0.8	7.0
4638		ANRC		440	9.9			0.9	1.7	16.8
4684				349	8.1			0.9	0.8	6.4
4603	0	1000	4	448	12.3	33.4	74.8	0.8	1.8	22.4
4641		ANRC		412	11.7			0.7	1.1	13.1
4608				417	8.8			0.7	0.9	8.3
4645	I	basal	1	462	16.5	25.7	9.1	0.6	0.1	1.2
4646				420	7.3			0.8	0.1	0.6
4674				563	12.3			0.6	0.3	2.8
4662	I	basal	1	494	10.6	32.5	15.3	0.9	0.3	3.3
4640				592	13.1			1.0	0.4	5.5
4685	II	1000	1	581	15.7	43.0	20.7	0.9	0.5	7.5
4614		alf.		571	18.6			0.6	0.3	4.7
4624	II	1000	1	624	14.6	63.1	51.3	1.1	0.6	9.2
4637		alf.		486	10.4			1.1	0.7	6.8
4686	III	2000	1	630	17.4	78.6	66.3	1.0	1.4	23.7
4620		alf.		598	18.8			0.8	1.4	25.5
4619	III	2000	1	434	14.1	75.6	83.1	0.7	2.5	34.7
4661		alf.		535	11.4			1.0	1.8	20.2
4690	IV	500	1	556	12.7	48.3	73.0	0.8	1.6	20.2
4665		alf. & 500		552	16.1			0.5	0.7	10.8
4628	IV	ANRC	1	569	11.6	44.7	55.4	0.7	0.8	9.5
5357				543	14.5			1.0	1.0	13.7

TABLE A3 (Continued)

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids μg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids μg/g liver	Vit A units/ g liver	Vit A units/ liver
4606	V	1000	1	601	12.2	63.1	123.4	1.3	3.0	36.5
4663		alf. & 1000		523	15.3			0.7	1.8	27.4
4673	V	ANRC	1	463	-*	55.6	99.7	-	-	-
4609				604	13.0			1.0	2.5	32.6
4696				571	13.6			0.7	1.8	24.1
4639	VI	1000	1	552	12.1	25.7	70.8	0.4	1.9	22.9
4695		ANRC		584	13.0			0.8	2.2	29.1
4657	VI	1000	1	698	15.3	27.4	73.4	0.7	2.5	37.8
4675		ANRC		473	14.7			0.4	1.3	18.8
4664	I	basal	2	698	12.7	32.5	7.7	0.5	1.1	13.4
4623				612	14.0			0.4	0.9	12.5
4671	I	basal	2	616	11.6	33.4	25.4	0.4	1.1	12.6
4631				687	16.8			0.4	0.7	11.6
4613	II	1000	2	502	11.8	43.8	31.1	0.4	0.9	10.2
4618		alf.		660	13.7			0.6	1.2	16.8
4659	II	1000	2	656	14.4	50.1	40.4	0.6	0.8	12.1
4683		alf.		737	16.4			0.9	1.2	19.1
4605	III	2000	2	500	15.1	79.5	85.3	0.7	1.1	16.7
4629		alf.		608	17.5			1.1	1.3	23.3
4652	III	2000	2	651	19.3	99.2	128.4	1.1	2.5	47.9
4634		alf.		702	14.8			1.1	2.5	37.0

TABLE A3 (Continued)

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids μg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids μg/g liver	Vit A units/ g liver	Vit A units/ liver
4621	IV	500	2	655	15.2	37.7	78.3	0.9	2.2	22.5
4700		alf. & 500		679	15.9			0.9	1.2	18.7
4627	IV	ANRC	2	598	13.6	41.2	71.7	0.5	1.2	15.6
4649				669	16.8			0.8	1.1	17.6
4633				634	13.9			0.7	1.3	18.3
4653	V	1000	2	548	13.4	63.1	110.6	0.8	1.2	15.4
4604		alf. & 1000		624	13.7			0.9	1.7	23.0
4642	V	ANRC	2	628	18.2	43.0	115.0	0.9	1.9	33.7
4697				686	14.8			1.0	3.1	45.3
4644	VI	1000	2	752	17.7	10.2	74.3	0.8	2.1	36.5
4630		ANRC		639	15.7			0.7	3.6	56.2
4625	VI	1000	2	674	15.8	30.8	100.6	0.6	1.8	27.7
4660		ANRC		669	18.9			0.5	1.1	20.0

[1]Four weeks pretest diet followed by 1 week and 2 weeks test diet.

[2]Serum samples obtained from pooled blood of 2 or 3 chicks as grouped in table.

*Liver not analyzed.

TABLE A4

Results of Serum and Liver Analysis
of Experiment IV

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids µg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids µg/g liver	Vit A units/ g liver	Vit A units/ liver
4513	A[3]	1000	4	402	9.7	66.9	64.6	1.2	0.7	6.6
4512		alf.		442	12.0			1.3	0.7	8.3
4523	A	1000	4	393	8.6	48.3	58.7	1.3	1.1	9.4
5324		alf.		481	9.3			0.8	0.5	4.6
5327	B[4]	1000	4	463	7.9	32.5	75.0	0.4	1.8	14.3
5336		ANRC		445	12.8			0.4	1.1	14.6
5346	B	1000	4	423	12.7	27.4	80.6	0.5	1.3	16.5
4503		ANRC		421	9.3			0.6	0.8	7.1
5306				475	9.2			0.6	0.5	4.8
5312	AI	basal	1	500	13.1	16.7	16.2	0.4	0.4	5.0
4531				510	14.9			0.4	0.4	6.0
5315	AI	basal	1	555	11.2	35.9	22.4	0.4	0.3	3.8
5344				510	12.2			0.6	0.4	4.4
5318	AII	1000	1	603	14.6	54.7	75.9	0.6	0.8	11.5
5313		alf.		527	11.1			0.7	0.8	8.7
4515	AII	1000	1	530	12.6	54.7	81.8	0.7	0.7	9.1
5320		alf.		526	11.0			0.7	0.9	9.6
5325	AIII	1000	1	563	13.9	32.5	83.8	0.5	0.6	7.9
5316		ANRC		564	11.2			0.8	1.6	17.5

TABLE A4 (Continued)

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids µg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids µg/g liver	Vit A units/ g liver	Vit A units/ liver
4506	AIII	1000	1	517	12.5	33.4	104.6	0.5	1.3	16.2
5326		ANRC		553	14.8			0.6	0.8	12.4
5335	BI	1000	1	584	12.7	43.0	78.5	0.8	2.1	27.0
5350		alf.		527	12.6			0.8	0.7	8.8
4508	BI	1000	1	588	13.1	48.3	68.7	0.8	1.5	19.6
4537		alf.		508	15.2			0.8	0.9	14.3
4532	BII	1500	1	606	12.8	55.6	81.6	0.8	1.9	23.9
5319		alf.		553	16.2			0.6	0.8	13.5
5304	BII	1500	1	483	9.6	61.2	95.4	0.7	0.9	9.0
4510		alf.		558	14.1			0.7	2.3	32.6
5311	BIII	1000	1	569	11.3	32.5	148.3	0.5	3.2	35.7
5337		ANRC		533	10.2			0.6	1.5	15.7
4512	BIII	1000	1	530	14.8	29.1	107.2	0.5	2.2	32.4
5329		ANRC		467	9.6			0.4	1.1	10.2
5317	AI	basal	2	573	16.3	46.5	63.3	0.4	0.3	4.1
4528				539	14.2			0.4	0.3	4.4
5334	AI	basal	2	457	13.5	51.9	55.2	0.4	0.1	1.3
4527				536	15.8			0.5	0.1	1.3
5323	AII	1000	2	563	14.0	33.4	20.3	0.7	0.9	15.0
5314		alf.		562	15.1			0.7	1.0	14.5
5338	AII	1000	2	507	13.2	21.6	2.6	0.7	0.3	4.5
4511		alf.		545	16.2			0.8	0.5	8.0
4505	AIII	1000	2	546	15.1	25.7	57.5	0.4	1.0	14.6
4525		ANRC		502	12.3			0.2	0.7	8.9
5349	AIII	1000	2	495	15.7	24.1	77.0	0.2	2.3	35.5
5341		ANRC		443	13.1			0.6	1.1	14.9

TABLE A4 (Continued)

Chick No.	Group No.	Type of diet	Duration diet[1] (weeks)	Body wt (g)	Liver wt (g)	Carotenoids µg/100 ml ser.[2]	Vit A units/ 100 ml serum[2]	Carotenoids µg/g liver	Vit A units/ g liver	Vit A units/ liver
5345	BI	1000	2	537	13.4	42.3	57.4	0.5	0.5	6.1
4514		alf.		574	17.3			0.8	1.5	28.1
5302	BI	1000	2	502	15.3	51.9	62.2	0.8	1.1	16.8
5301		alf.		523	13.7			1.0	1.2	15.7
5339	BII	1500	2	540	12.0	68.8	90.7	0.9	1.0	11.6
5343		alf.		600	14.7			1.2	5.7	83.4
5307	BII	1500	2	576	15.7	55.6	54.3	0.9	1.8	28.3
5332		alf.		527	20.2			0.6	0.5	9.1
4519	BIII	1000	2	527	15.7	26.6	104.6	0.6	2.7	42.4
5308		ANRC		582	14.7			0.4	1.4	20.4
4529	BIII	1000	2	426	12.8	22.4	51.9	0.3	1.5	18.7
4502		ANRC		480	13.0			0.2	0.6	8.2

[1]Four weeks pretest diet followed by 1 week and 2 weeks test diet.

[2]Serum samples obtained from pooled blood of 2 or 3 chicks as grouped in table.

[3]Divided into groups AI, AII and AIII for test diet.

[4]Divided into groups BI, BII and BIII for test diet.

A STUDY OF NUTRIENT CONDITIONING. UTILIZATION OF BETA CAROTENE OF NATURAL SOURCES BY CHICKS PREVIOUSLY GIVEN VITAMIN A per se

by

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AN ABSTRACT OF A MASTER'S THESIS

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An attempt was made to determine whether chicks could be so conditioned to the use of pure vitamin A that they would utilize beta-carotene from alfalfa meal less efficiently after they had been fed pure vitamin A for a certain length of time. Serum and liver vitamin A levels were used as the criteria of vitamin A utilization. This investigation was based on 4 experiments.

Newly hatched chicks were fed a vitamin A deficient diet for 1 week to deplete most of their maternal vitamin A reserves. They subsequently were fed 1000 IU vitamin A potency per kg of feed for 4 weeks (the pretest diet). In 3 of the 4 experiments, i.e. experiments I, II and III, vitamin A in the pretest diet was supplied by the ANRC vitamin A standard. In experiment IV one group of chicks received the vitamin A activity from the ANRC vitamin A standard and another group from alfalfa meal. The purpose of feeding beta-carotene previous to feeding the test diet was to obtain additional information on the effect of type of pretest nutrient upon the utilization of subsequently fed beta-carotene.

At the end of the 4-week pretest period chicks were randomized into several groups. Each group was fed graded levels of vitamin A activity supplemented to the diet through ANRC vitamin A standard, alfalfa meal, or a mixture of both vitamin A sources.

Several chicks from each group were sacrificed at the end of the pretest period and after chicks had been fed the test diet for 1 week or 2 weeks. The sera and livers were assayed for vitamin A.

Though results were not entirely consistent, decreased values of serum and liver vitamin A generally were observed after beta-carotene was substituted for an equivalent number of units of pure vitamin A fed in the

pretest diet. This was possibly due to a conditioning effect of pure vitamin A.

Weight gains and feed conversion ratio data from this work generally indicated that chicks could utilize equivalent vitamin A potencies from pure vitamin A and alfalfa source with the same degree of efficiency to promote growth. No additional information regarding conditioning effect could be obtained from these data.

It is suggested that further investigations should be conducted in nutrient conditioning since results were not entirely consistent, and this field has many aspects that have not been studied.