Influence of Different Forms of Vitamin A, Thyroxine, and Thiouracil on Carotenoid Utilization by Chicks* (32992)

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The adverse effect of high dietary levels of vitamin A on the utilization of carotenoids by the chick has been amply demonstrated (1, 2). Vitamin A was found to interfere with the absorption of these pigments from the intestinal tract (2).

Several studies have suggested an association between vitamin A and the activity of the thyroid gland. It has been reported that the increased metabolic rate observed in thyroxine injected rats could be partially counteracted by the simultaneous administration of vitamin A (3). A decreased metabolic rate and thyroid size in rats has been reported as a result of administration of large amounts of vitamin A (4). Recently, it has been shown that the increased fragility of erythrocytes from chicks fed an excess of vitamin A may be an indirect effect of suppression of thyroid activity (5).

These experiments were conducted to study the influence of different forms of vitamin A on carotenoid utilization and to determine if the effect of vitamin A on the utilization of these pigments is mediated through the thyroid gland. For the latter purpose, vitamin A was fed in combination with thyroxine or thiouracil.

Materials and Methods. Five-week-old male Vantress X Arbor Acre chicks were used. The chicks had previously been fed a carotenoid free diet (a white corn–soybean meal starting diet). They were housed as groups in rearing batteries with raised wire-screen floors. Feed and water were supplied *ad libitum.* Individual body weights were obtained at the beginning and end of the test periods. Feed consumption records were obtained by groups.

The composition of the basal diet was similar to the one used in the first experiment of a previous study (2), with the exception that chromic oxide was omitted and an equal amount of yellow corn was added. Yellow corn was the dietary source of carotenoids. The dietary treatments in experiment 1 were formulated by supplementing the basal diet with different vitamin A compounds. Vitamin A palmitate[2] was added at 5000 and 100,000 IU/kg of diet; retinol,[3] retinal,[4] retinoic acid[5] and 13-*cis*-retinal[6] were used at 100,000 IU/kg of diet. Vitamin A palmitate was mixed directly in the feed, all other forms of vitamin A were dissolved in vegetable oil[7] before they were added to the diets. The mixed diets were stored in a cooler to minimize losses of carotenoids or pure vitamin A compounds due to oxidation.

In the second experiment, the basal diet was supplemented with vitamin A palmitate at 5000 and 100,000 IU/kg of diet. The other dietary treatments were formed by adding L-thyroxine sodium or thiouracil to each of the above vitamin A levels. Thyroxine was added to the diet at the rate of 6.6 mg/kg whereas thiouracil was fed at 0.4% of the diet and was included at the expense of soybean meal.

In both experiments, duplicate groups of birds (10/group) were fed the experimental diets for a 3-week period.

At the termination of the test period, the birds were bled by cardiac puncture, killed by disjunction of the neck, and the livers and

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[2] Vitamin A palmitate in gelatin, 250,000 IU/gm Addition to feed based on declared potency. From Hoffmann-La Roche, Nutley, N. J.

[3,4,5,6] Eastman Organic Chemicals No. 5159, 8080, 8098, and 8082, respectively. Additions to feed based on their theoretical potencies.

[7] Wesson Oil, distributed by Wesson Oil Sales Company, Fullerton, California.

TABLE I. Effect of Different Forms of Vitamin A on the Performance and Utilization of Carotenoids (Expt. 1).

	Av wt. (gm)			Carotenoids[b]		
Forms of vitamin A (IU/kg)	Initial (5 week)	Final (8 week)	Feed/gain[a]	Serum[c] (μg/ml)	Liver[c] (μg/gm)	Toe-web skin[c] (μg/100 cm^2)
Vitamin A palmitate, 5000	779	1424	2.26	4.3^{e}	2.9^{e}	83^{e}
Vitamin A palmitate, 100,000	817	1509	2.18	1.2^{f}	0.9^{fg}	41^{f}
Retinol, 100,000	781	1448	2.27	1.5^{f}	1.1^{f}	46^{f}
Retinal, 100,000	791	1440	2.32	1.1^{f}	0.8^{fg}	32^{f}
Retinoic acid, 100,000	789	1360	2.48	0.8^{f}	0.6^{g}	26^{f}
13-*cis*-Retinal, 100,000	790	1474	2.21	1.0^{f}	0.7^{fg}	37^{f}
SE[d]			±0.04	±0.29	±0.08	±3.37

[a] Feed-to-gain ratio during the experimental period.

[b] Each value is the average of 4 separate determinations (samples from 5 birds were pooled for each determination).

[c] Means within each column having different superscripts are significantly different: $p < .01$.

[d] Standard error of the general mean.

feet were removed. In experiment 2, thyroid glands were removed from some of the birds from each treatment and their weights were recorded. Within each dietary group, serum, liver, and toe-web skin samples from each of 5 birds were pooled for determniation of carotenoids as described previously (2). Statistical examinations of the data were made using analysis of variance, and treatment differences were separated using a multiple range test (6).

Results and Discussion. Table I shows the results of feeding different vitamin A compounds on growth performance and utilization of carotenoids. Final body weight and feed-to-gain ratio during the experimental period were depressed in the group fed retinoic acid as compared with the other dietary groups. Supplementation of diets with 100,000 IU of vitamin A/kg regardless of the form of the vitamin, significantly depressed ($p < .01$) carotenoid levels in serum, liver and skin as compared with the group containing 5000 IU of vitamin A/kg of diet. There was variation in the carotenoid lowering effect of different forms of vitamin A. However, significant difference was obtained only in the liver carotenoids of the group fed retinoic acid, which had significantly less ($p < .01$) carotenoids than the group fed retinol. The retinoic acid fed group also exhibited a greater decrease of serum and skin carotenoids as compared with other groups.

The results from the above study indicate that a change in the terminal functional group of vitamin A does not affect the carotenoid depressing effect of this vitamin. In a similar study, Wood (7) found no relationship between the end structure of vitamin A and its hypocholesterolemic action.

The structure–activity relationships of vitamin A have been studied at great length and recently reviewed by Ames (8). It has been reported that retinoic acid is not stored in the body in any appreciable amount. This would suggest that the inhibitory effect of retinoic acid on carotenoids would take place mainly in the intestinal tract. This would tend to confirm a previous report (2) where high vitamin A caused a decrease in the absorption of these pigments.

The second experiment was conducted to determine the effect of vitamin A in combination with thyroxine or thiouracil on the utili-

TABLE II. Effect of Vitamin A, Thyroxine, and Thiouracil on the Performance, Utilization of Carotenoids, and Thyroid Weights (Expt. 2).

Dietary treatments (IU/kg)	Initial (5 week) (gm)	Final (8 week) (gm)	Feed/gain^a	Carotenoids^b Serum^c (μg/ml)	Liver^c (μg/gm)	Toe-web skin^c (μg/100 cm^2)	Thyroid wt. (mg)
Vitamin A, 5000	842	1471	2.51	3.8^f	2.3^f	68^f	84 ± 26 (16)^d
Vitamin A, 100,000	830	1439	2.48	0.8^g	0.7^{gh}	38^{gh}	78 ± 25 (16)
Vitamin A, 5000 + thyroxine	846	1428	2.62	3.8^f	2.7^f	63^f	53 ± 14 (15)
Vitamin A, 100,000 + thyroxine	795	1362	2.79	1.1^g	0.8^{gh}	35^h	37 ± 12 (12)
Vitamin A, 5000 + thiouracil	818	1332	2.72	3.4^f	1.2^g	53^{fg}	116 ± 45 (20)
Vitamin A, 100,000 + thiouracil	806	1322	2.68	0.6^g	0.3^h	31^h	107 ± 44 (20)
SE^e			±0.08	±0.17	±0.15	±2.93	

[a] Feed-to-gain ratio during the experimental period.

[b] Each value is the average of 4 separate determinations (samples from 5 birds were pooled for each determination).

[c] Means within each column having different superscripts are significantly different: $p <.01$.

[d] Mean wt. ± SE; number of glands weighed is given in parentheses.

[e] Standard error of the general mean.

zation of carotenoids. The results are shown in Table II. The final body weights in the thiouracil fed groups were lower than the other dietary groups. Feed-to-gain ratio during the experimental period was not significantly affected by different dietary treatments.

Serum carotenoids were significantly depressed ($p < .01$) by the higher level of vitamin A supplementation. Liver and skin carotenoids in the groups fed 100,000 IU of vitamin A/kg of diet were significantly lower ($p < .01$) than the groups fed 5000 IU of vitamin A/kg of diet, with the exception of the group which was also supplemented with thiouracil. Thyroxine supplementation resulted in slight increases of serum and liver carotenoids at both vitamin A levels, but did not affect skin carotenoids.

Thiouracil at the lower vitamin A level caused a significant decrease ($p < .01$) in the liver carotenoids whereas at the higher level of vitamin A supplementation, thiouracil produced a nonsignificant decrease in liver carotenoids. Also serum and skin carotenoids were depressed by thiouracil, but these differences were not statistically significant as compared with their corresponding vitamin A fed groups.

As would be expected, the weights of thyroid were increased by thiouracil supplementation and were decreased in the groups fed thyroxine (Table II). The higher level of vitamin A supplementation did not appear to influence thyroid size. This is in contrast to the work with rats (4), where a decrease in thyroid size by high vitamin A administration was observed.

The effect of thiouracil and high vitamin A on carotenoids appears to be somewhat similar. However, these data do not suggest a possible indirect effect of vitamin A on thyroid in depressing carotenoid utilization. It would still appear that vitamin A mainly interferes with the absorption of these pigments (2), possibly by its direct effect in or on the intestinal tract. Obviously, there may be other mechanisms by which vitamin A influences the utilization of carotenoids.

Summary. Forms of vitamin A having alcoholic, aldehydic, or acidic terminal functional groups, when fed at 100,000 IU of vitamin A/kg of diet, depressed carotenoid utilization. The retinoic acid caused the decrease in the carotenoid content of serum, liver, and skin to a greater extent than other forms of vitamin A. In an experiment designed to study the effect of vitamin A in combination with thyroxine or thiouracil on the utilization of carotenoids, a similarity of their effects between high vitamin A and thiouracil was evident.

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Zinc Content of Human Platelets (32993)

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Comparison of the zinc concentration in 16 samples of plasma and serum obtained at the same venipuncture in 14 normal individuals revealed an increase of 16% zinc in serum after clotting (1). The source of this greater zinc content could be from one of the cellular elements which are destroyed during the coagulation process. While zinc in erythrocytes and in leukocytes has been determined (2–6), the presence of zinc in platelets has not been demonstrated (7). Since platelets are largely disintegrated in the process of clotting (8), zinc would be released if it were present. Other factors might include dilution (more volume of plasma than of serum per aliquot of whole blood), and hemolysis. The purposes of these experiments were to explain the difference in the zinc levels between plasma and serum, and to determine whether zinc is present in platelets in a significant amount.

Material and Methods. Nine healthy males aged 25–62 and one female with thrombocytosis each supplied 100 ml of venous blood. This was drawn using a 19-gauge needle[2], through polyethylene tubing into a Fenwal no. 1682[3] polyethylene blood pack unit containing 15 ml of acid citrate dextrose (ACD) as the anticoagulant. Constant, gentle mixing was required. The ACD solution was shown not to be contaminated by zinc. This sample was used for isolating platelets. All samples were collected between 9 and 10 a.m. Preparation of zinc-free glass and plastic ware has been previously described (1).

The 100-ml blood sample was transferred from the plastic donor bag to a 250-ml centrifuge tube and allowed to stand for 1 hour at 4°C prior to differential centrifugation for

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[3] Fenwal Laboratories, Morton Grove, Illinois.