ACCUMULATION AND EXCRETION OF VITAMIN A-LIKE FLUORESCENT MATERIAL BY SEBACEOUS GLANDS AFTER THE ORAL FEEDING OF VARIOUS CAROTENOIDS*

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In our earlier work (1, 2), we injected various carotenoids intradermally and visualized them by their auto-fluorescence within the sebaceous complexes in the vicinity. Carotene and vitamin A were characterized operationally based upon their auto-fluorescence in the formalin fixed biopsy specimens: (a) Carotene was characterized by its yellow-gold, slow-fading fluorescence. (b) Vitamin A and/or a vitamin A-like material was characterized by a brilliant green fading fluorescence (3).

The vitamin A-like material was accumulated within the cells of the sebaceous glands, within the sebum in the hair follicles, and in the interstices of the stratum corneum. Based on these direct observations on normal individuals and on patients in whom the follicles were plugged, we hypothesized an external route in the skin for vitamin A.

Normally, neither vitamin A nor carotene is present in detectible amounts by the standard chemical or histologic methods (4, 5, 6). In this paper we will report the physiologic accumulation of the vitamin A-like material in the same external route via the sebum in man subsequent to the chronic ingestion of carotene; while failing subsequent to the chronic ingestion of equivalent or greater amounts of vitamin A. The serum carotene and vitamin A values were determined. It is noteworthy: (a) That the vitamin A-like fluorescence was present in the sebum and in the epidermis in proportion to the enhanced serum carotene values. (b) That a carotene tolerance developed as judged by chronic serum carotene values.

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METHODS

Serum carotene and vitamin A determinations (7) were made on the initial day of the carotenoid regimen and at monthly intervals thereafter.

The individuals tested were either out-patient or hospital patients of the dermatology division at Cook County Hospital. They did not present absorption defects; they suffered from a variety of usually localized dermatoses not thought to be associated with vitamin A deficiency.

Punch biopsy specimens (8 mm.) from apparently normal skin were taken from the back usually at the time when the blood was drawn. The tissues were fixed (4 hours) in 10% formalin. Frozen sections (20 microns in thickness) were made and examined microscopically for their autofluorescence according to the method, somewhat modified (2), of Popper and Greenberg (3).

Results A. Group 1

The feeding experiments fell into three groups. Group 1 consisted of 2 patients: (a) One was fed small doses (equivalent to 10,000 U.S.P. units of vitamin A daily) of a Carotene Concentrate in Oil[1] preparation. (b) The other was fed carrot juice[2] containing carotene equivalent to 180,000 U.S.P. units daily. Within one to two months we observed: (a) The serum carotene concentration was increased 3 to 4 fold to values of 500–750 gammas per cent. (b) The green fading fluorescence was visualized in high intensity (4 plus) within the sebaceous gland cells, the sebum, the stratum corneum and the rete mucosum. (c) The serum vitamin A values remained essentially unchanged. (d) There were no clinical signs of vitamin A toxicity.

It is noteworthy that the vitamin A-like fluo-

[1] Carotene Concentrate in Oil. Wyeth Laboratories Inc., Phila. Pa. Derived from the non-saponifiable fraction of vegetables oils. 5000 U.S.P. (equivalent) Vitamin A per capsule.

[2] Eveready Carrot Juice. Hawaiian Pineapple Co., Ltd., San Jose, Calif. Carotene content approximately 5,100 I.U. (equivalent) vitamin A per fluid ounce.

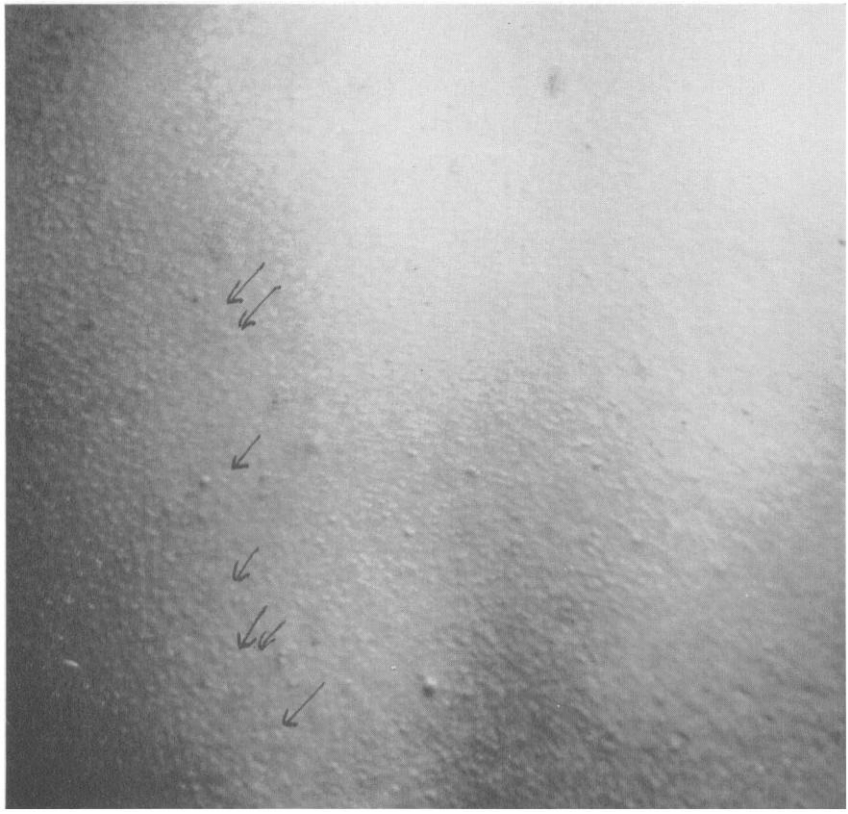

FIG. 1. *Subject in whom carotenoderma was developed.* In this black and white photograph, the bright green fluorescent hair follicles stand out as white dots. The photograph was made while the subject's back was being irradiated with an ultra-violet Wood's lamp.

rescence seen histologically was readily observed at each of the hair follicles of the intact skin (See Figures 1 and 2). This macroscopic green fluorescence was observed in only these two subjects. Further there was a typical but slight carotenoderma in these subjects only. Histologically this was associated with yellow gold fluorescent flecks characteristic of carotene within the stratum corneum and within the dermis just beneath the tips of the papillae. In addition there was a salmon to brilliant red fluorescent material within the follicles and at the neck of the sebaceous glands. A red fluorescent material could be expressed from the follicles at this time and for months thereafter.

Results B. Group 2

The chemical data for the remaining subjects are summarized in Table 1. Carotene Concentrate in Oil,[1] the non-saponifiable fraction of vegetable oil; Roche Beta Carotene,[3] a crystalline carotene preparation; and Research Carrot Oil,[4] a carrot oil extract, were fed. The serum carotene values remained essentially unchanged throughout the carotene ingestion period in the 9 subjects who received 5000–10,000 U.S.P. units daily of the Carotene Concentrate in Oil.[1] The serum carotene values were increased by 50% to 150% above the initial values within one month with all the carotene preparations within the dose range from 20,000 to 100,000 U.S.P. units daily. However, the cumulative rate of rise of the serum carotene values was either markedly diminished or re-

[3] Roche Beta Carotene. Kindly supplied by Hoffman-La Roche, Inc. Nutley, N. Jersey. A crystalline preparation of beta carotene. (12 mgm./capsule)

[4] Research Carrot Oil. Kindly Supplied by Nutritional Research Associates, Inc. South Whitley, Indiana. The fat soluble fraction of the carrot root. Standard potency: 25,000 U.S.P. (equivalent) vitamin A per gram. (0.8 gm. per capsule)

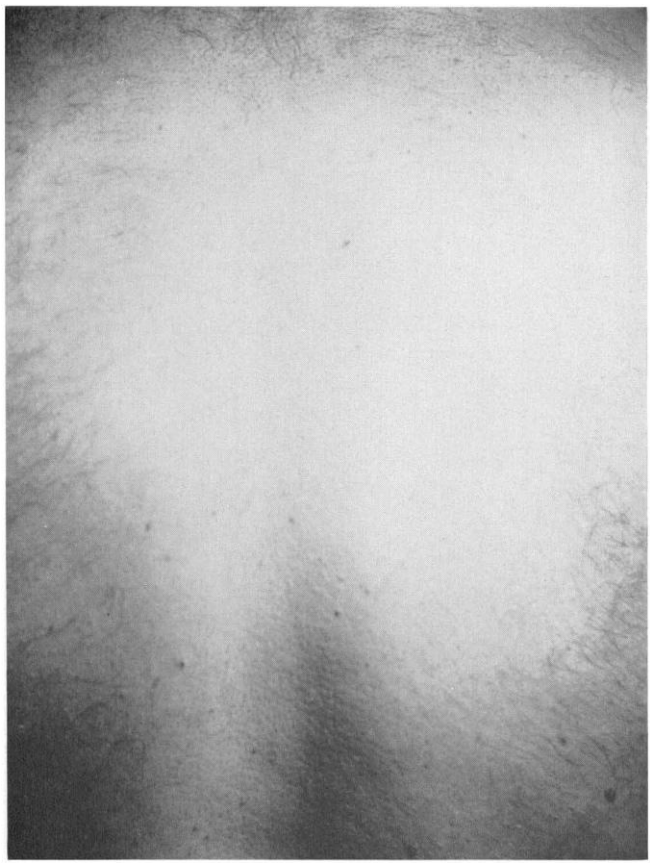

FIG. 2. *Normal subject for comparison.* This photograph was made as in Figure 1; the hair follicles were not fluorescent.

versed in two thirds of the subjects in the second and third months.

Actually, a decreased serum carotene concentration was seen in the second month: (a) in 9 of 19 of the subjects receiving the various doses of Carotene Concentrate in Oil, (b) in 6 of 15 of the subjects receiving Roche Beta Carotene, and (c) in 2 of 5 of the subjects receiving the Research Carrot Oil. The serum carotene concentration showed a small percentage increase in the second month: (a) in 3 of 19 of the subjects receiving the Carotene Concentrate in Oil, (b) in 4 of 15 of the subjects receiving the Roche Beta Carotene, and (c) in 2 of 5 of the subjects receiving the Research Carrot Oil.

Essentially after one month, there were obtained three characteristic serum carotene curves: (a) a slowly rising curve in about ⅓ of the subjects, (b) a plateau curve in about ⅓ of the subjects, and (c) a slowly decreasing curve in about ⅓ of the subjects.

The serum carotene curves obtained were remarkably low and remarkably similar despite the wide range of doses administered.

The serum vitamin A values (see Table 1) remained essentially unchanged throughout the carotene ingestion period. There were no clinical signs of vitamin A toxicity (8).

Generally, the initial biopsy specimens were devoid of either of the autofluorescent carotenoids. In several instances trace intensities of the green fading fluorescence were visualized within the sebaceous glands. At the end of the first month of carotene feeding, the green fading fluorescence was visualized within the cells of the sebaceous glands in trace to 1 plus intensities in nearly every instance. At two months, the green fading fluorescence was in heightened intensity (1 plus to 2 plus) within the sebaceous glands. None of

TABLE 1

Serum carotene and vitamin A concentration in gamma % in 44 subjects during chronic carotenoid ingestion

Dose/Day (Equivalent to Vit. A in I.U.)	No. of Subjects		Initial	1 Month	2 Months	3 Months
Roche Beta Carotene[3], 100,000.	15	Serum Carotene	128 (±68)	308 (±116)	313 (±141)	314 (±184)
		Serum Vit. A	64 (±18)	71 (±9)	74 (±11)	82 (±17)
R.C.O.[4] 40,000.	5	Serum Carotene	113 (±47)	187 (±76)	194 (±101)	
		Serum Vit. A	69 (±16)	80 (±31)	74 (±21)	
Carotene concentrate in oil,[1] 40,000.	5	Serum Carotene	118 (±34)	241 (±95)	283 (±78)	
		Serum Vit. A	72 (±9)	76 (±38)	72 (±11)	
Carotene concentrate in oil,[1] 20,000.	5	Serum Carotene	159 (±54)	262 (±69)	—	288 (±119)
		Serum Vit. A	65 (±19)	103 (±17)	—	88 (±44)
Carotene concentrate in oil,[1] 5000–10,000.	9	Serum Carotene	139 (±95)	139 (±67)	—	104 (±28)
		Serum Vit. A	72 (±17)	65 (±9)	—	60 (±7)
Roche Vit. A Acetate,[5] 100,000.	5	Serum Carotene	87 (±39)	87 (±42)	—	
		Serum Vit. A	85 (±28)	153 (±108)	—	

the Group 2 subjects demonstrated the green fading fluorescence within the sebum, the interstices of the stratum corneum, or the rete mucosum as did the two subjects of Group 1. Neither did we observe the yellow gold fluorescence of carotene within any of these sites. However, small amounts of the unidentified salmon to red fluorescent material, as described for Group 1, were frequently observed within the follicles in the biopsy material after the second month of carotene ingestion.

Results C. Group 3

Similarly, a group of 5 subjects were fed vitamin A acetate[5] (100,000 U.S.P. daily) for one month. At one month the serum vitamin A values were increased from 50% to 200% while the serum carotene values remained essentially unchanged. Further, the sebaceous glands contained zero to trace intensities of the green fading fluorescent material except in one subject in whom we observed the fluorescence in moderately increased intensity. In no instance was the green fading fluorescence observed in the sebum or in the stratum corneum; nor did we observe the red fluorescent material within the hair follicles as with the subjects of Groups 1 and 2. The above histologic findings were equally true for several additional patients not listed in Table 1 who were fed three times to ten times the above dosage of vitamin A acetate for 2–3 months and longer.

[5] Vitamin A Acetate. Kindly supplied by Hoffmann-La Roche Inc., Nutley, N. Jersey. (50,000 I.U./capsule).

DISCUSSION

1. Carotene Hypothesis

Carotene is universally considered to be present in normal skin in the stratum corneum although the experimental evidence is meager and questionable (9, 10, 11). Several authors have alluded to its presence in sweat glands (12, 13, 15, 16, 17) or in sebaceous glands (13, 14, 15, 16, 17); to our knowledge neither carotene nor vitamin A have been demonstrated histologically or chemically either in these structures or their secretions (4, 5, 6, 10).

In our data, in the two subjects (Group 1) in whom the serum carotene values were raised 3–4 fold (500–750 gammas%) we observed the vitamin A-like fluorescence in high intensity within the sebum and within the entire epidermis and none within the sweat glands or ducts. In the other 39 subjects (Group 2) in whom the serum carotene values were raised 1 to 2 fold or less (200–300 gammas %), the vitamin A-like fluorescence was visualized in decreased intensity within the sebaceous glands only. The vitamin A-like fluorescence was not seen within the sebum when equivalent or greater amounts of vitamin A were ingested. We therefore hypothesize that the carotene rather than the vitamin A of the serum serves as the principal carotenoid for the sebaceous complex.

Vitamin A supplementation has been much used in therapy in dermatology; there are almost no reports (18) of the use of carotene supplements. Recently, in a symposium on nutritional disease,

Scrimshaw (19) reported remarkably low serum carotene values (44–85 gammas %) associated with serum vitamin A values (20–29 gammas %) that were within normal limits in school children in 3 Central American countries (except Guatemala). Follicular hyperkeratosis was present in 45–55% of these children, and it remained unchanged despite vitamin A supplements (40,000 I.U. daily for 14 weeks) or fat supplements (48 grams of lard, etc., 5 days a week for 56 weeks). In view of our hypothesis we would predict that carotene supplementation would benefit these school children; although it is conceivable that the hypocarotenemia in this population is the result of a generally enhanced carotene tolerance as discussed below.

2. Carotene Tolerance

A carotene tolerance, as judged by the serum carotene values, was developed with the chronic ingestion of carotene in 39 of 41 subjects. This phenomenon, though surprising to us, was described previously, although sketchily and on few patients, by Kaufman and Drigalski (20) and Wendt (21). And recently in an excellent work, Urbach *et al* (24) obtained a two fold increased serum carotene concentration which remained unchanged during a 6 month period of massive carotene ingestion in 4 subjects. Urbach's conclusion that the body does not have control mechanisms to regulate the level of serum carrotene seems unjustified, even though vitamins A, C and E are regulated more efficiently.

Other authors have commented on the great variability amongst individuals in the development of carotenemia (13, 14, 15, 22). Where case histories have been obtained in patients with carotenoderma, it usually required from 4–12 months or longer of supplementary carotene (12, 14, 15, 17); however among the patients there were described several individuals in whom 1-2 months or less sufficed (14, 17, 22).

Significantly, we observed a several fold increase in the peak carotene values associated with carotenoderma in only 2 of 41 subjects. Probably, there is a previous natural selection in the clinical studies so that only the carotenoderma susceptible individuals present themselves to the physician.

Further, in our study and in Urbach's (24), the serum vitamin A values remained essentially unchanged throughout the carotene ingestion period. Thus there is no direct evidence for the concept that the low peak carotene values are related to an accelerated rate of conversion of carotene to vitamin A. Neither is this mechanism ruled out for vitamin A blood levels are known to be closely regulated (24). Significantly, despite the enormous carotene intake for several months there were none of the classical signs of vitamin A toxicity in our subjects (8, 23).

SUMMARY

1. A vitamin A-like fluorescent material was visualized within the sebaceous glands and in the sebum and throughout the epidermis in the 2 of 41 subjects in whom the serum carotene values were increased 3 to 4 fold by long term carotene supplementation.

2. In the remaining 39 of 41 subjects the vitamin A-like fluorescence was visualized in the sebaceous glands only and the serum carotene values were increased only 1 to 2 fold or less.

3. Vitamin A acetate in 3 to 10 times equivalent doses did not induce the vitamin A-like fluorescence within the sebum and the epidermis.

4. Based on these findings we hypothesized that the vitamin A-like material visualized in the sebum is derived principally from the serum carotene and not from the serum vitamin A.

5. Carotene tolerance as judged by the serum carotene values was developed in almost all of the subjects. This was discussed in relation to the variability in susceptibility to carotenoderma.

6. The above carotene tolerance may in part be the mechanism to explain the freedom from toxicity observed even with massive carotene alimentation.

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DISCUSSION

DR. VICTOR R. WHEATLEY (Chicago, Ill.): The one point I should like to make here is that there is still no real evidence that the material is vitamin A. Giving the best judgment possible on your work, we can say that the carotenoids are converted into a green fluorescent substance, but I think it is premature to assume that it is vitamin A. Biologically the conversion of β-carotene to vitamin A is a complex process and not the simple piece of scissors biochemistry it appears on paper. I can't understand why when you feed large amounts of vitamin A you do not get it in the sebaceous gland. If it was vitamin A I would expect the reverse to be true.

DR. RUVEN GREENBERG: I agree that we do not have positive proof that the green fading material is vitamin A. I was very careful to call it a vitamin-like material. I, too, agree that the increase of the fluorescence in the sebum does not come about by feeding vitamin A, rather it was produced by feeding carotene, and that's the whole point of this paper.

Originally, with the intradermal injection of a solubilized carotene, we demonstrated that this vitamin A-like material travels within the sebum via an external route to the stratum corneum. In this paper we were able to reproduce this route physiologically, in 2 of 41 patients in whom we raised the serum carotene level 3 to 4 fold while at the same time the serum vitamin A values remained essentially unchanged. This makes me feel that the serum carotene is the precursor of vitamin A; or at least it is the precursor of a vitamin A-like material within the sebum.

DR. THEODORE CORNBLEET (in closing): We were rather disappointed when we could not duplicate the high levels of blood carotene attained in the earlier experiments.

Three patients were given vitamin A first, and then given carotenes afterward; in those three we attained much higher levels of serum-carotene and also a larger amount of carotene and vitamin A in the sebaceous glands. In the present experiments we did not administer vitamin A either before or during the feeding of carotene. Perhaps this may explain the modest rises in serum-carotene levels we are reporting.

One thing stands out in the clinical work—there are no ill effects, no untoward results from the giving of the large doses of any of the preparations of carotene we employed. This is in contrast to what could happen from the use of comparable doses of vitamin A. If there is a place for pharmacological doses of vitamin A in dermatology, then perhaps carotene could replace the vitamin, at least for greater safety.