AN ABSTRACT OF THE THESIS OF

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TITLE: Vitamin A Status of Vegetarians

ABSTRACT APPROVED: ________________________
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In order to determine the vitamin A status of vegetarians, 142 volunteers aged 15-45 were asked to record their diets for three consecutive days and provide fasting plasma samples. Forty-six per cent ate meat less than once a week (vegetarians), 28% one to four times a week (moderate omnivores), and 26% more often than that (omnivores). Among the three groups of women the mean total daily vitamin A intake ranged from 10,377 to 11,622 IU and the mean plasma concentration from 44.1 to 49.0 ug%, all with large standard deviations. Among the men the differences were greater but likewise not statistically significant at a 5% level, probably due in part to substantial variation. The mean intakes among the three groups ranged from 8,189 to 12,237 IU and mean plasma concentrations from 51.9 to 59.8 ug%. Women who took vitamin A supplements had virtually identical mean dietary intakes and mean plasma concentrations of the vitamin as those who didn't. Men who used supplements had slightly lower intakes and higher blood levels. Vegetarians and moderate omnivore women obtained about a third of their dietary vitamin A from carrots alone. Although the moderate omnivores of both sexes tended to have higher plasma levels of vitamin A, no basis was seen for concluding that vitamin A status differed significantly among the three dietary groups into which the participants were classified. A recently

introduced spectrofluorometric micromethod for measuring plasma vitamin A was afflicted by unexpected fluorescent interference in some of the samples, especially from women, and hence should be used cautiously in the future for surveys such as this one.

VITAMIN A STATUS OF VEGETARIANS

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INTRODUCTION

Human beings undoubtedly have been following multifarious diets for as long as homo has been sapiens and hungry. "Vegetarian" was first coined to identify members of a society of non-meat eaters founded in Manchester, England, in 1847. About the same time in the United States, Ellen B. White was organizing the Seventh-Day Adventists from thousands of disappointed, destitute millenarians at Battle Creek, Michigan, where she denounced animal flesh as a desecration of God's temple, human flesh. Today, the 250,000 Seventh-Day Adventists who follow a lacto-ovo-vegetarian diet constitute the largest association of United States vegetarians (1).

There are no reliable estimates of the total number of non-meat eaters presently in this country. The last pertinent scientific poll, conducted during World War II, claimed 2.5-3.0 million (2). The North American Vegetarian Society believes there are now between five and ten million (3).

"Vegetarianism" embraces several different dietary practices. Vegans refrain from eating any animal foods and sometimes from using products prepared with animal material. Lacto-vegetarians include dairy products in their meals while lacto-ovo-vegetarians eat eggs as well. Fruitarians rely solely upon fruits and granivores upon seeds and grains (1). Fruitarians, granivores, and vegans comprise a small minority of occidental vegetarians.

The nutritional status of vegetarians residing in an affluent society such as ours, where access to a year-round agricultural abundance is regarded as a virtual right, has seldom been examined. During the social upheaval of the last decade in America, the tasting of other lifestyles incited shrill polemics against radical diets but few dispassionate surveys of "new vegetarians", so-called because of their non-affiliation with earlier U.S. sects.

Meanwhile, epidemiological data have begun to appear during the past few years relating lower dietary intakes of vitamin A to the incidence of some epithelial cancers in humans, supporting laboratory demonstrations that susceptibility to certain carcinogenic insults in animals depends in part on vitamin A status (4,5,6). Aside from liver, the richest and most varied sources of the vitamin are vegetables and fruits. Perhaps a difference in vitamin A status may help to explain why one vegetarian group in the United States enjoys a dramatically lower mortality rate for certain cancers suspected of being related to dietary habits (7).

Since published research on the vitamin A intake of non-meat eaters is scarce and not always useful and since information on the levels of the vitamin circulating in their blood is not available, a study of the vitamin A status of vegetarians was undertaken.

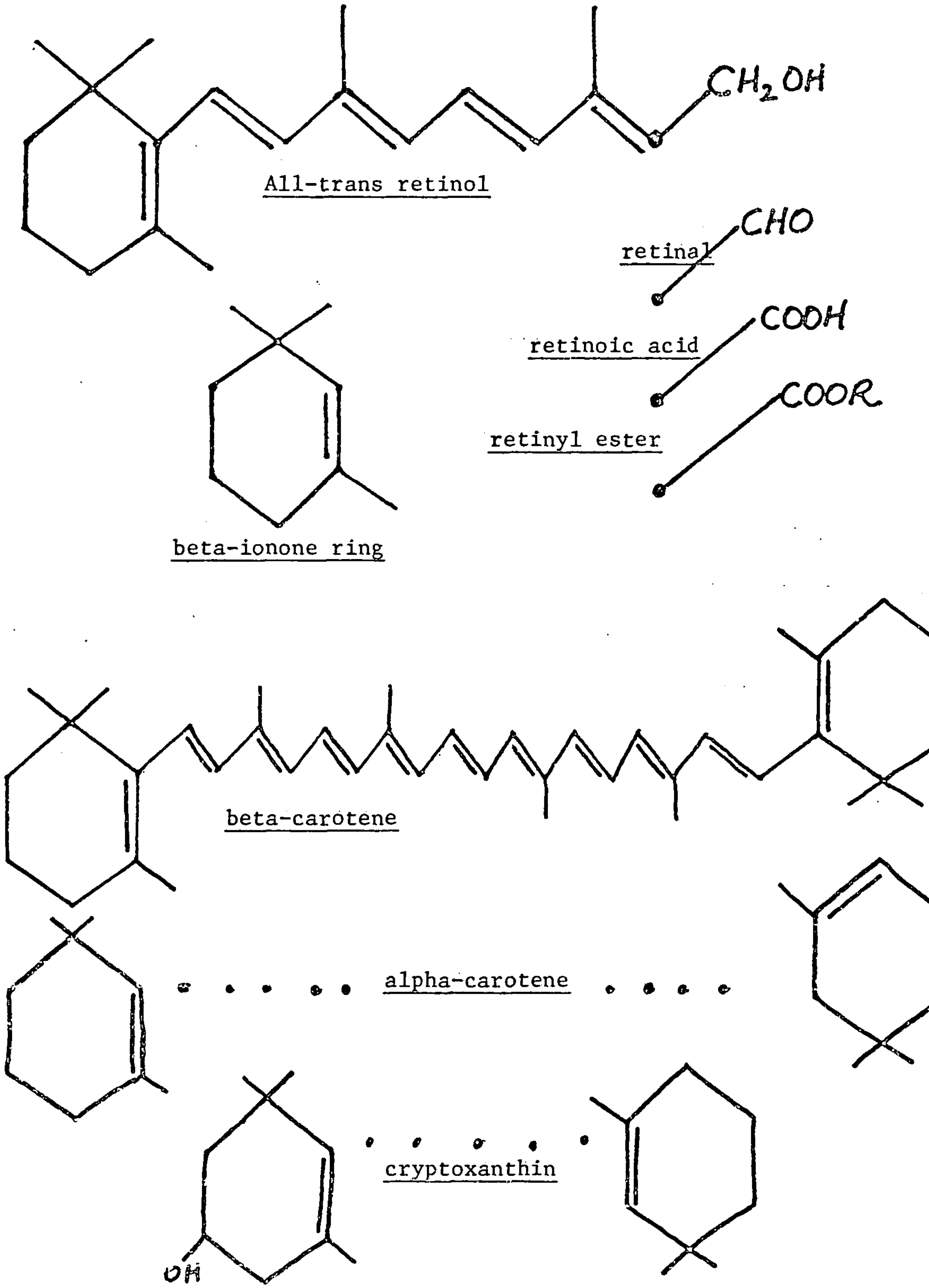
VITAMIN A

"Vitamin A" is a general term denoting all the substances structurally and biologically related to retinol. This includes both the "provitamin" carotenoids, precursors of retinol synthesized by plants, and the "preformed vitamin A" compounds --- retinol, retinal, retinoic acid, and retinyl esters --- which are physiologically active in vertebrate animals. Plants are the ultimate source for all vitamin A since vertebrates cannot synthesize de novo either carotenoids or retinol but can only convert the former into the latter. Conversely, plants do not transform any of their carotenoids into the preformed vitamin (8).

CHEMISTRY

The structures of the most important vitamin A molecules are presented in Figure 1, page 4. An unmodified beta-ionone ring is essential for vitamin activity (9). The biologically most potent conformation of the unsaturated hydrocarbon chain, all-trans, is thought to be the principal form in food and blood (9,10). Any other stereoisomer, with the exception of 11-cis retinal in the visual cycle, has greatly diminished or insignificant vitamin activity. Some of the terminal functional groups are interconvertible. Retinol, the vitamer normally circulating in the plasma, can be reversibly oxidized to an aldehyde, retinal, the active form in the retina and an intermediate in the digestion of provitamins. Retinal, in turn, can be irreversibly oxidized to retinoic acid, which is capable of functioning as the vitamin at all tissue sites but the retina and reproductive organs (11). Vitamin A is stored in animal tissue as the retinyl esters of saturated fatty acids, usually palmitate.

FIGURE 1


CH2OH
All-trans retinol
CHO
retinal
COOH
retinoic acid
COOR
retinyl ester
beta-ionone ring
beta-carotene
alpha-carotene
cryptoxanthin
OH

Carotenoids are a large family of terpenoid yellow-orange pigments, found in virtually every living organism on earth, whose polyene hydrocarbon chain provides much of the beauty in nature. The colors of green foliage, flower petals, flamingoes, lobsters, and goldfish, for example, emanate in part from carotenoids. The splendor of autumn unfolds when they blaze through the decomposing chlorophyll molecules of dying leaves and are oxidized through an array of dazzling pigments (12).

Although more than 300 carotenoids have been characterized thus far, less than ten have any provitamin activity and only three are significant in the human diet (8,13). By far the most important of these three is beta-carotene because it is widely distributed in vegetables and fruits and because it possesses two of the essential beta-ionone rings (see Figure 1). In contrast, alpha-carotene, which often accompanies it in plant tissues, and cryptoxanthin, the "yellow" of corn, have only one unmodified beta-ionone ring and are accordingly rated at half the vitamin value of beta-carotene (14).

FUNCTIONS

Carotenoids function as accessory pigments in photosynthetic organisms, relaying radiant energy to the primary pigments and buffering chlorophyll from photo-oxidation (15). Chlorophyll is always accompanied by carotenoids. Indeed, the green color of plant tissue results from the blending of chlorophyll's blue-green with the yellow-orange of carotenoids; that is why the degree of greenness is a rough index to the concentration of vitamin A in certain plants.

Despite intensive scrutiny for more than 60 years, the functions of preformed vitamin A in vertebrates remain, in the words of J.A. Olson, "tantalizingly mysterious" (9). What is manifest is that vitamin A deficiency is one of the most serious and lethal of dietary inadequacies, leading to night blindness, keratinization of epithelial and hyper-keratinization of epidermal tissues, failure of appetite, cessation of growth, weight loss, blindness, infection, reproductive disorders, defective bone modeling, nerve damage, and finally death --- usually from infection or renal obstruction (13). Such consequences are seldom found in the well-nourished industrial nations but mainly afflict poorer countries, particularly in the Western Hemisphere and Asia, which rely on starchy foods with little carotenoid content (16).

The sole function of the vitamin clearly understood is its role in night vision, as elucidated by George Wald. There the central event is the isomerization of 11-cis retinal to all-trans retinal by a photon of light in the rod cells of the retina, creating an electric potential relayed to the brain (17). Presently, much research is focused on the involvement of vitamin A with epithelial cell differentiation. In the vitamin's absence, mucociliated cells are sloughed off and replaced by basal squamous cells coated with keratin while the goblet cells of the intestinal tract become considerably diminished. Similar keratinization of the conjunctiva and cornea results in blindness. Vitamin A is now suspected of transporting glycosyl residues for the biosynthesis of membrane glycoproteins (18).

The Food and Nutrition Board of the National Academy of Sciences bases their Recommended Dietary Allowances (RDA) on the quantity of vitamin A necessary to avoid the earliest signs of deficiency (14). Night blindness can be avoided with a minimum of 150 ug of retinol daily, while an additional 450 ug will prevent follicular hyperkeratosis of the skin and sustain vitamin A above the hazardous threshold in the plasma (19). To provide for hepatic storage, which does not occur with a marginal intake, the daily allowance is increased to 1000 ug or 5000 International Units (IU) for the adult male and 800 ug retinol or 4000 IU for the adult female. One microgram of retinol is equal to 3.3 IU of vitamin A or to 6 ug of beta-carotene (14).

DIETARY SOURCES

Vitamin A is available from food as the provitamin carotenoids of plant tissue, as the retinyl esters in animal flesh or in the nutrient reserves for their young (eggs, milk), and as the synthetic retinyl esters of commercially fortified products (dairy foods, margarine, breakfast cereals, and soy protein foods). Aside from liver and kidneys, the richest natural sources of vitamin A are vegetables and fruits, followed by dairy products and eggs which contain moderate amounts. Muscle tissue, especially pork, and fish are not good sources. See Table 1, page 8.

About 8100 IU of vitamin A per person per day were available in the U.S. food supply in 1976, an amount which has remained fairly constant throughout the century (20,21). A distribution of sources for 1971-2 is presented in Table 2, page 8. Dietary allowances are based on the assumption that in the United States half is consumed as preformed vitamin and half as provitamin (14).

Vitamin A is quite stable in most stored foods and remarkably little, compared to many other vitamins, is lost with processing and home cooking. Only in powdered dehydrated foods exposed to oxygen can

TABLE 1

ANY OF THE FOLLOWING QUANTITIES OF FOOD WILL SATISFY THE U.S. RECOMMENDED DIETARY ALLOWANCE OF VITAMIN A FOR AN ADULT MALE. FOR A TYPICAL MIXED DIET THE ALLOWANCE IS 5000 IU. HOWEVER, BECAUSE OF VARYING DIGESTIBILITY AND BIOLOGICAL ACTIVITY, FOR AN ENTIRELY PRE-FORMED VITAMIN A DIET IT IS EQUIVALENT TO 3,333 IU AND FOR AN ALL-PROVITAMIN A DIET 10,000 IU (14,56).

1/5	oz	beef liver	3.3	oz	carrots
10	oz	beef kidney	0.5	c	sweet potato
6		eggs	2/3	c	spinach
2.4	qt	whole milk	1.8	c	cantaloupe
13	oz	American cheese	2.6	c	broccoli
21	lb	ground beef	12		small tomatoes
67		chicken drumsticks	2/5		pumpkin pie
26	c	tuna fish	10		apricots
∞	lb	pork	1.4	c	winter squash

TABLE 2

PERCENTAGE CONTRIBUTION OF FOODS TO U.S. VITAMIN A SUPPLY, 1971-2 (76)

Vegetables	35.0
Meat	22.4
Dairy Products	13.1
Fats	8.1
Fruit	7.4
Eggs	6.5
Potatoes, sweet potatoes	5.0
Miscellaneous	2.3
Starch, cereals	0.4
Legumes, sugar	0.0

severe losses be expected (10).

DIGESTION AND ABSORPTION

Since the carotenoids and vitamin A are fat-soluble, their digestion and absorption depend upon a sufficient quantity of lipid in the diet as well as adequate pancreatic secretions and bile salts for emulsification (11). The preformed vitamin is usually present in food esterified to a long chain fatty acid. This ester bond is cleaved either by a pancreatic esterase in the lumen of the small intestine or by a hydrolase at the mucosal brush border. The retinol products are then actively absorbed into the mucosal cells where they are rapidly re-esterified, preferentially with plamitate or stearate (22). The RDA is based on the assumption that 100% of the dietary preformed vitamin is assimilated (14).

Carotenoids, on the other hand, are not as efficiently absorbed, probably because of a lower solubility in lipids, and most are excreted unchanged with the feces (23). The remainder are admitted into the intestinal mucosa where some of the provitamins are apparently snapped in half by 15,15' beta-carotene dioxygenase employing molecular oxygen. The retinal molecules thus formed are reduced by a nonspecific reductase and esterified to fatty acids (22). Still to be satisfactorily explained is the fact that beta-carotene has seldom been observed experimentally to yield stoichiometrically more than one molecule of retinol (11). The Food and Nutrition Board calculated their allowances on the supposition that only one-third of dietary provitamins are absorbed and that one beta-carotene molecule yields only one net retinol molecule. Thus, 6 ug of dietary beta-carotene are thought necessary to equal 1 ug of of retinol (14). There is evidence that, depending upon the source and medium, considerably more than one-third of the provitamins may actually be absorbed. Rao and Rao reported, for example, that carotenoid absorption from carrots ranged from 16% to 67% in four subjects and that efficiency of absorption increased as the beta-carotene content of food increased (24).

TRANSPORT AND STORAGE

The retinyl esters and unmodified carotenoids in the human intestinal mucosa are incorporated into the chylomicrons and transported to the general circulation via the lymph system, bypassing the liver. Humans are unique among mammals in this regard because most species, including the common laboratory animals, accept little or no carotenoids into their systems while a few species, such as cows, horses, and sheep, admit only certain ones. Guernsey and Jersey cows, more adept at accumulating carotenes than other breeds, are renowned for the consequent orangish hue of their milk (25).

Within minutes after the chylomicrons spill from the thoracic duct near the right shoulder they are cleared from the bloodstream by adipose tissue and the liver. Carotenoids are subsequently deposited throughout the body and seem to be absent only from sweat, spermatozoa, and cerebrospinal fluid. Their accumulation in fat depots colors the white of infant fat yellow by adulthood (25). The retinyl esters are hydrolyzed upon uptake by the liver, re-esterified, and stored in the parenchymal and Kupffer cells. The capacity of the liver, which holds at least 80% to 90% of the vitamin A reserves, is enormous and under all but the most extreme circumstances impossible to saturate with dietary vitamin A. A representative adult male in the United States has stockpiled roughly 525,000 IU in his liver, or about 135 times the amount circulating in the blood, equivalent to the vitamin A ingested from 32 pounds of carrots(13).

Vitamin A and carotenoids rely upon protein in order to traverse aqueous blood. Carotenoids accompany lipoproteins, mostly in the cholesterol-rich low-density lipoprotein fraction. Carotenemia, the circulationtion of such an excessive quantity of carotenoids that deposits in subcutaneous fat stain the skin orange, does not result in hyperlipidemia since the lipoproteins appear to possess ample binding capacity (26); nor does it result in hypervitaminosis A since little if any circulating carotenoids are converted to the preformed vitamin (13).

Retinol travels in a 1:1 molar ratio with a specific transport protein, retinol binding protein (RBP), which, in turn, forms a highly specific and stable complex with a prealbumin two and one half times its own molecular weight. First characterized in 1968, RBP is synthesized in the liver and enjoys a half-life in the blood of about 12 hours (27). Its secretion is depressed during certain diseases, especially hepatic, and with protein-calorie malnutrition, apparently reflecting diminished protein synthesis. With vitamin A deficiency alone, apo-RBP collects in the liver from where it is rapidly mustered when the vitamin is once again available. Notwithstanding its obvious importance, the mechanism for hydrolysis of stored retinyl esters and their non-covalent attachment to RBPs for release into the bloodstream is still unknown. A suitable esterase candidate has been isolated only once, in 1966, and subsequent attempts by other researchers to duplicate this work have failed (27).

VITAMIN A STATUS

The concentration of retinol circulating with RBP in the blood is the most frequently used and practical biochemical measure of vitamin A status, the capacity of an individual to satisfy his or her metabolic requirements for the vitamin (28). Its significance, however, must be carefully evaluated in light of the vitamin A equilibrium between blood and the liver and the many circumstances which affect it.

Liver reserves will sustain the plasma concentration of vitamin A at a level characteristic for an individual independently of short-term fluctuations in vitamin A intake. Even as the stores are being exhausted without replacement from the diet, the plasma concentration usually will not decline until the hepatic supply is seriously depleted. Consequently, a low plasma level in the absence of malnutrition or hepatic disease is indicative of low reserves, but a "normal" plasma concentration is not necessarily a guarantee of adequate stores (29). In a World War II experiment with conscientious objectors which serves as the basis for the daily requirements recommended by the United Kingdom and the World Health Organization of the United Nations, plasma vitamin A levels did not fall significantly for eight months in any of 16 subjects consuming only traces of the vitamin; one 27 year old man exhibited no appreciable change after 22 months (23).

On the other hand, a steady plasma level also means that a brief, uncharacteristic indulgence in the vitamin will not necessarily conceal the quantity ordinarily circulating. Even sporadi ingestion of synthetic vitamin supplements does not usually produce a lasting effect (13). Whether or not longer term consumption patterns are ultimately reflected in the blood is an important question which will be addressed later.

Among the factors reported to influence the concentration of plasma retinol are (a) sex, (b) age, (c) reproductive cycle and pregnancy, (d) season, (e) illness, especially with fever, (f) gastrointestinal disorders, and (g) drugs.

Adult males have levels about 20% higher than adult females while the latter circulate slightly more carotenoids. After physical maturation there is no consistent relationship to age until at least the fifth decade of life when levels appear to rise slightly (30,13). In women, plasma vitamin A is elevated during the half of their menstrual cycle following ovulation and lowest during menses (31, 32); it also falls in the first trimester of pregnancy, climbs during the second and third, then plummets shortly before childbirth (33). No diurnal pattern is evident and seasonal variation, more pronounced for carotenoids, has sometimes but not always been detected for vitamin A (13).

Lower plasma levels have been noted with cardiac disease, kidney ailments, cancer, tuberculosis, pneumonia, and other infections as well as with gastrointestinal disorders such as celiac disease or biliary tract obstruction. Fever can depress the circulating concentration by 50% (11), while oral contraceptives can elevate it up to 50% in women (34). Sedatives and anticonvulsant medication have recently been reported to raise plasma retinol levels in humans (35).

VEGETARIANS

The most recent survey of scientific literature on "non-flesh dietaries" appeared in 1963 (36). Nearly all the group studies to that date described either the consequences of some unusual eating habits practiced in uncivilized areas of the world or the impact of the civilized nations' wars on the health of its victims. Only two investigations focused on vegetarians living in an industrial nation at peace. Mirone studied six Trappist monks subsisting on meager fare in Georgia (37); and Hardinge and Stare, in still the most comprehensive study of U.S. vegetarians, examined the nutritional status and health of 112 subjects during the early 1950s (38).

In other relevant research which has also considered vitamin A, Oldham and Sheft followed the nitrogen balance of 13 pregnant U.S. women, seven of whom were lacto-ovo-vegetarians (LOV) (39), and Guggenheim and associates investigated the diets of 119 Israeli vegans of all ages (40). A 1967 review of the nutritional status of vegetarians in the United Kingdom, of whom there were thought to be 100,000, included data from a study of 26 LOV (41). The diets of 57 New England adherents of "Zen macrobiotics", who strive for a balance in their lives and diets inspired by Oriental religion, were examined by Brown and Bergan (42,43, 44). Their 1975 abstract provides the only published hint of plasma vitamin A levels in vegetarians (44). Finally, Harland and Peterson recently assessed the diets of 16 LOV Trappist monks in Maryland who were suspected of consuming mineral-deficient diets (45).

Table 3 on page 15 presents the mean daily vitamin A intakes reported by the five U.S. studies. The British and Israeli investigations,

TABLE 3

MEAN DAILY VITAMIN A INTAKES (IU) FROM FIVE UNITED STATES STUDIES OF VEGETARIANS, 1951-1978, TOGETHER WITH A 1972 SAMPLE OF THE GENERAL POPULATION.

MALES

GROUP	n	IU	REFERENCE
Macrobiotics	3	5860	Brown & Bergan (42)
Ga. Trappists	6	8131	Mirone (37)
Md. Trappists	16	8684	Harland & Peterson (45)
Macrobiotics	29	11,824	Brown & Bergan (43)
Lacto-ovo-vegetarian	15	15,540	Hardinge & Stare (38)
Vegans	14	25,570	Hardinge & Stare (38)
HANES survey of general population		5125	HANES (30)
Control omnivores	15	14,420	Hardinge & Stare (38)
RDA		5000	RDA (14)

FEMALES

GROUP	n	IU	REFERENCE
Macrobiotics	4	5010	Brown & Bergan (42)
Pregnant lacto-ovo	7	8046	Oldham & Sheft (39)
Macrobiotics	21	9313	Brown & Bergan (43)
Lacto-ovo-vegetarian	15	13,470	Hardinge & Stare (38)
Vegans	11	19,510	Hardinge & Stare (38)
HANES survey of general population		4027	HANES (30)
Control omnivores	15	13,730	Hardinge & Stare (38)
RDA		4000	RDA (14)

which reported means of 6800 IU and 7289 IU, respectively, are omitted because their failure to discriminate among ages and sex makes interpretation difficult. Included for comparison are preliminary data from the Health and Nutrition Examination Survey (HANES) of 1971-2 conducted by the U.S. Department of Health, Education and Welfare using a scientifically designed sample of 10,126 to estimate the nutritional status of the entire U.S. population (30).

All groups met or exceeded their RDAs for vitamin A and each vegetarian group seemed to consume more than the corresponding HANES sample. That vegetarians might be consuming more vitamin A than omnivores is not startling in view of the best sources of the vitamin. Hardinge and Stare's research indicates that they could be deriving 50% more of their calories from vitamin A-rich foods, which is especially significant because many of these foods are low in energy value. Table 4 on page 17 contrasts the distribution of calories for their 88 meat eaters and 86 LOV. The differences reflect more than just the replacement of meat with other protein sources. Male vegetarians, for instance, consumed not only 25% more calories from dairy products and eggs but also 86% more from fruits and vegetables, 50% less from sweets, and 25% less from visible fats as well. Potentially more than one-quarter of the calories from a meat diet, representing flesh, fat, and sugar, may be subject to substitution in the LOV diet.

Yet, whether or not vegetarians actually do have higher daily vitamin A intakes than omnivores cannot be conclusively answered from the reports cited in Table 3 because of the following complications.

First, although vitamin A consumption can vary substantially with

TABLE 4

PERCENTAGE DISTRIBUTION OF CALORIES IN DIETS OF 88 OMNIVORES (O) AND 86 LACTO-OVO-VEGETARIANS (LOV). (38)

FOOD	O	LOV	% DIFFERENCE
	MALES		
Meat	12.9	0.0	100 %
Dairy products,eggs	15.9	19.7	24 %
Vegetables,fruits	11.5	21.4	86 %
Cereals,starches,legumes, soups	23.1	38.4	66 %
Fats	10.8	8.2	24 %
Sweets	25.0	11.9	52 %
Total of vegetables,eggs, fruit,dairy products	27.4	41.1	50 %
	FEMALES		
Meat	12.4	0.0	100 %
Dairy products,eggs	13.2	22.0	67 %
Vegetables,fruits	12.5	19.5	56 %
Cereals,starches,legumes, soups	23.6	28.4	20 %
Fats	12.3	13.1	7 %
Sweets	24.0	16.3	32 %
Total of vegetables,eggs, fruits,dairy products	25.7	41.5	61 %

the seasonal availability of fruits and vegetables, most of these studies did not indicate when their data were collected. Only in Mirone's investigation of six Trappist monks was the difference noted: vitamin A intake was 37% higher from April through September than during the remainder of the year (37). The seven pregnant women were followed for three to four months during their second and third trimesters, the macrobiotics compiled ten-day records, and the 16 other Trappists recalled one day of meals, but the season was not specified in any of these reports, making comparisons uncertain.

Second, in Hardinge and Stare's study, which comprises 40% of the vegetarian data, the omnivores consumed not only about the same amount of vitamin A as the LOV but also far more than the vegetarians in any of the other studies and nearly three times as much as the HANES subjects.

Third, one-quarter of the male vegetarians were Trappists, the most penitential of all Catholic monks, who did not choose their own menus and are not necessarily representative of other American vegetarians. Six were living on a spartan diet consisting largely of coarse bread and barley coffee (37).

Fourth, the two reports on Zen macrobiotic enthusiasts differed in their results by about two-fold for both males and females, perhaps owing to seasonal differences. Moreover, 14% of the subjects in the larger study were omnivores (43).

Fifth, the seven pregnant women could have (and should have) been consuming more nutrients than was their usual custom and so many not be illustrative of other vegetarian women who, of course, are usually not pregnant (39).

Accordingly, the first purpose in studying the vitamin A status of free-living American vegetarians is to establish what their intake is and if it exceeds that of a comparable meat eating population during the same season of the year.

If vegetarians do indeed constitute a group with a higher mean intake of vitamin A, would that be reflected in higher levels of the vitamin circulating in their blood? Since that question has yet to be answered (or apparently asked), this is the second purpose for studying the vitamin A status of vegetarians. No group of healthy Americans other than males has thus far been discovered with consistently higher plasma concentrations of retinol.

In the only pertinent report on U.S. vegetarians to surface in the literature, Brown and Bergan found that vitamin A was the one major nutrient present at acceptable levels in the blood of all 94 of their macrobiotic subjects, but no details were reported (44). Patwardhan, reviewing the nutritional status of the Third World, noted a positive correlation of 0.762 $\pm$ 0.316 between the plasma concentration and the dietary intake of vitamin A for ten countries who derived 60% to 100% of their retinol from provitamins. Nigeria, which had the highest intake at 7500 IU daily, also exhibited the highest plasma retinol level (46). Dagadu and Gillman reported from Ghana, where carotene-rich foods like palm oil and mangoes are consumed, that women there had serum levels of vitamin A greater than women in European countries and that one-third of the women and one-fifth of the men tested showed quite high blood levels. No specific intakes were recorded (47).

In Britain, Leitner and his co-workers noted a general upward trend for plasma vitamin A concentration over a ten-year period concomitant with an increased consumption of vitamin A-rich foods in that country (48). The Ten-State Nutrition Survey in the United States detected a slight tendency for plasma retinol to rise with increasing dietary levels of the vitamin for certain segments of the population, but researchers were unwilling to draw firm conclusions because of the considerable variability (49).

A third reason for studying the vitamin A status of vegetarians is that susceptibility to carcinogenic insult may depend in part on the vitamin A status of the host. Vitamin A is essential for the normal differentiation of epithelial tissue where well over half of the total primary cancer sites occur in both men and women in the United States (6). Rats deficient in the vitamin are more vulnerable to respiratory, bladder, and colon cancers than those sufficient in retinol. In 1974 Bjelke reported that of 4885 newly diagnosed human cases of stomach, colon, or rectal cancer in Norway and in Minnesota, the colon and rectal patients had significantly lower intakes of fish, vegetables, and vitamin A than their matched, non-cancer controls. In Norway, carrots were the vegetables that most distinguished the diets of control patients from those with colon cancer (4). A year later Bjelke reported from a five-year survey of more than 8000 Norwegian men that vitamin A intake was negatively correlated with the incidence of lung cancer in all age groups and at all levels of cigarette smoking (5). Low blood vitamin A levels have been noticed in one group of lung cancer patients (50), but not in another (51).

Moreover, ever since the discovery in 1966 that excess vitamin A could protect hamsters from carcinomas of the stomach and cervix, experimental evidence has been building which demonstrates that under some circumstances vitamin A can prevent the development of epithelial cancer in vivo and can even reverse, at least in vitro, the course of lesions already induced by chemical carcinogens (6). One vitamin A analog, 13-cis retinoic acid, which protected hamsters from a carcinogenic challenge that induced tumors in 10% of the controls, is currently being tested on 100 human subjects in the U.S. at great risk of bladder cancer (52).

A recent eight-year study of 35,460 Seventh-Day Adventists aged 35 or older living in California discovered that the men suffered a cancer mortality rate only 53% and the women 67% of that of the general population. When this group was compared with another non-smoking but non-Adventist sample, mortality rates for cancer of the colon, breast, ovary, and prostate remained significantly lower. Since many dietary factors in addition to vitamin A, such as levels of saturated fat, vitamin C, and fiber, may also be involved in the etiology of cancer, a six-year prospective study is currently in progress to relate dietary practices to site-specific cancer incidence rates in 100,000 California Adventists (7).

METHODS

SUBJECTS

Subjects were recruited from the general campus population with newspaper advertisements and public notices and from among students enrolled in an upper division nutrition course. Vegetarians were encouraged to participate but all interested were welcomed and promised a detailed computer analysis of their diets, undoubtedly the major incentive for volunteering.

Participants were informed in writing of the general nature of the study but not the specific vitamin to be examined, and were requested to:

(a) record their diet for three consecutive days, following instructions and forms provided. A three-day instead of a one-day record was selected because vitamin A intake tends to fluctuate more than other nutrients (53), and because the protocol of another study from which additional subjects were obtained (see below) also specified three days.

(b) answer questions about age, sex, height, and weight; frequency of consumption of animal products; incidence of serious illness during the preceding three months; use of vitamin supplements; use of oral contraceptives or other drugs.

(c) on the morning of the fourth day, before eating breakfast, allow a licensed medical technician to draw a small amount of blood from their arms. Women were asked to schedule this to occur during the second half of their menstrual cycle.

The participants were assured of anonymity and of their right to withdraw from the study at any time and were asked to sign informed consent statements in accordance with the regulations of the Oregon State

University Board for the Protection of Human Subjects, which authorized this research on September 22, 1978.

One hundred and twenty-three persons recorded their diets and appeared for blood drawing during the three weeks of the study. In addition to these campus volunteers, 24 three-day records and plasma samples collected during the same time were obtained from an on-going investigation of vegetarians in the local community (54).

Five of the 147 subjects were excluded from the dietary computations because their three-day records were inadequate. Of the remaining 142, 22 were excluded from the plasma comparisons because:

(a) blood could not be drawn from one and samples from two others hemolyzed;

(b) one was pregnant and another's menses began on the third day of the diet period;

(c) one was 15, another 16 years of age, ages usually not classified as adult in other surveys (30,49);

(d) three were recovering from typhoid, malaria, or infectious mononucleosis suffered during the preceding three months;

(e) three were taking either an anti-depressant, a diuretic anti-hypertensive, or a sedative medication;

(f) two were being treated for a thyroid condition which has previously been shown to affect the intestinal conversion of provitamins to retinol (11):

(g) seven suffered colds, some with fever, during the experimental period. The plasma retinol concentration of subjects in a published study plunged during the week a cold swept through their ranks (55).

DIETS

The vitamin A content of the diets in IU was calculated from food values in the U.S.D.A. Handbook No. 456 (56) and from information deposited in the Ohio State Nutrient Data Bank (57). No attempt was made to include vitamin supplements in the dietary data because too many subjects could not recall precisely the quantities they ingested.

BLOOD

A spectrofluorometric micromethod introduced in 1971 and refined in 1973 by J.N. Thompson and his colleagues at the Canadian Department of National Health and Welfare in Ottawa was chosen to measure vitamin A in the plasma (58,59). A micromethod was specifically sought in order to minimize the volume of blood necessary and not discourage any prospective volunteers. The most widely used assay for 50 years, a colorimetric method using the corrosive acid antimony chloride (60), is vulnerable to moisture and produces only a fleeting color, rendering it unsuitable for a microassay (27,61). A later modification substitutes trifluoroacetic acid to evolve a more stable color (62).

Both these assays encounter substantial interference from beta-carotene which also reacts with the Lewis acids. This is usually corrected for by later deducting the concentration of beta-carotene, obtained by attributing to it all the sample's absorbance at 450 nm in an organic solvent. McLaren, however, has demonstrated that this tactic may produce misleading results since many non-provitamin carotenoids also absorb at 450 nm (63). In the blood samples of vegetarians, who might reasonably be suspected of circulating substantial quantities of various carotenoids in their blood, this could be critical.

A spectrofluorometric method offers several advantages. First, since vitamin A is very fluorescent, very small concentrations, down to 0.01 ug/ml, can be readily detected, rendering it quite suitable for a micromethod. The intensity of fluorescence is strictly proportional to the concentration of retinol from 0.003 uM to 6.0 uM (64). The usual concentration of a microfluorometric vitamin A assay is about 1.75 uM.

Second, the use of paired wavelengths for activation and emission should increase specificity beyond that of spectrophotometry which relies on one wavelength setting (61).

Third, beta-carotene does not normally fluoresce at the same wavelengths with vitamin A. In fact, only the carotenoid phytofluene (discussed below) is thought to offer significant interference.

Fourth, the procedure is simple and quick and uses relatively inexpensive, non-corrosive organic reagents.

The major drawbacks in general to fluorometry are contamination by extraneous fluorescent matter and quenching by reagents. Plastic materials, such as tubing, must be avoided and blanks carefully monitored for stray fluorescence. Some laboratories find it necessary to redistill or chromatograph their reagents before use and some do not (64).

The major obstacle in the spectrofluorometric vitamin A assay appears to be interference by phytofluene, a non-provitamin carotenoid found in a variety of fruits and vegetables, which is quite readily absorbed by humans and circulates in the plasma at a concentration roughly 10% of retinol (65).

Phytofluene exhibits three main absorption peaks at about 331, 349, and 367 nm. Its fluorescence at an activation wavelength of 330 nm and

and emission wavelength of 480 nm coincides with the maximum fluorescence of retinol, so Thompson devised a correction formula to deduct the extra fluorescence due to phytofluene. Since the fluorescence of phytofluene is maximal at an activation wavelength of about 370 nm but retinol's is minimal, a constant ratio of fluorescence for purified phytofluene at 370 nm to that at 330 nm is first established. Then, each blood sample is measured at 330 nm to obtain a gross fluorescence and at 370 nm to learn the extent of phytofluene's presence; by means of the constant ratio the amount of fluorescence at 330 nm due to phytofluene can be calculated. Using this strategy, Thompson claimed results consistent with earlier colorimetric methods and with methods employing a preliminary chromatographic separation (59).

Unfortunately, the formula proved to be unsatisfactory in this study. Plasma vitamin A levels for a certain nine healthy subjects averaged a highly improbable 11.5 ug%, a concentration usually associated with clinical symptoms. Examination of their plasma's fluorescence spectra revealed a sharp peak at 350 nm whose effect spilled over to 370 nm and apparently exaggerated the fluorescence due to phytofluene. No common or unusual factor could be detected in the diet or habits of these nine subjects to explain this. Although their mean vitamin A intake was 25% higher than the other participants, the range was sevenfold. Eight of the nine were women, significant if the interferent was a carotenoid because women circulate higher quantities of carotenoids in their blood (13).

The retinol in these samples was isolated on 5% activated alumina (58,59) using Pasteur pipet columns (66). Recovery was 98%. The consequent mean concentration, assayed fluorometrically, was 51.3 ug%, four and a half times the product of the correction formula and almost identical to the mean of the other participants. Examination of other plasma samples revealed unusual but more modest fluorescence at 350 nm in about 7% of the subjects. All but one of these were women. Their vitamin A concentrations, calculated according to the correction formula, ranged from 24.2 to 68.7 ug%. The possibility thus remains that vitamin A levels could be underestimated in some cases becasue the fluorescence due to phytofluene may have been distorted.

Nevertheless, Thompson's technique was followed closely for 93% of this study's subjects and the results, discussed below, were consistent with other assays. The maximum fluorescence of retinol in an Aminco Bowman spectrofluorometer was recorded at an activation wavelength of 330 nm and an emission wavelength of 480 nm, while the maximum fluorescence of phytofluene was found at corresponding wavelengths of 370 nm and 480 nm. These were similar to wavelengths used by others (58,59,64, 65,67). Following Thompson, the widest slit width arrangement was used. Phytofluene was isolated from fresh tomatoes on a 2% activated alumina column and the ratio of fluorescence at 370 nm to that at 330 nm computed to be 1.34. Thompson recorded 1.38-1.42 (59).

Venous blood samples were drawn into heparinized Vacutainer tubes and centrifuged. Since vitamin A is vulnerable to light-induced isomerization and to oxidative degradation, the plasma was stored at -10 degrees Centigrade in small vials shielded from light with aluminum foil wrapping. Opinions on the stability of vitamin A under these conditions range from two days to one year (68,69,64).

Assays were conducted at night in dim light, usually within seven to ten days after the blood was drawn. The mean coefficient of variation of the duplicates was 5.8%, similar to that reported by some and much less than others (67,64). A control plasma sample, assayed at the beginning of each evening's run, averaged 65.4 ug%, with a standard deviation of ± 0.87. Such a small variation over a period of six weeks seemed to demonstrate that the subjects' samples were stable during the course of the experimental work.

Lacing the plasma with synthetic beta-carotene did not disturb fluorescence and recovery of retinol added to plasma was 94%.

STATISTICS

Means, standard deviations, correlations, and one-way analyses of variance were performed on a programmed Hewlett-Packard Model 10 Calculator (70). An alpha level of 5% was chosen to identify significance (71).

RESULTS AND DISCUSSION

The 142 dietary subjects are profiled in Table 5 on page 30. Based on their own estimates of meat consumption, they were assigned to one of three groups: (a) those who ate meat less than once a week were designated vegetarians (V); (B) those who customarily ate meat one to four times a week were called moderate omnivores (M-O): and, (c) those who consumed it more often than that, omnivores (O). Forty-six percent were accordingly classified V, 28% M-O, and 26% O.

The three groups of women were quite similar in mean age and height. V, however, weighed seven to twelve pounds less than the M-O and O; eight of the 92 women (9%) were overweight, judged by the Metropolitan Life Insurance Company's weight standards (72). V women were much more likely to be ingesting vitamin A supplements but less apt to be using oral contraceptives. Although the exact proportion of campus women using oral contraceptives is unknown, the best available estimate is about a quarter (73).

The mean age of male O was four years younger than that of V and M-O, yet mean heights and weights were similar. Four of the 50 (8%) were overweight. In contrast to the women, V men were the least likely to be relying on vitamin A supplements.

Mean daily vitamin A intakes are presented in Table 6 on page 31. All groups consumed much more than their RDAs. Although some variation existed among the males, there were no statistically significant differences in total vitamin A intake among V, M-O, and O for either men or women, perhaps owing in part to the substantial standard deviations. Male V consumed a significantly greater quantity of provitamins than

TABLE 5

PROFILE OF DIETARY SUBJECTS

	V	M-O	O	ALL
WOMEN				
Number	41	27	24	92
Mean age	23.9	24.2	24.6	24.2
Mean height (inches)	65.0	65.4	65.5	65.3
Mean weight (pounds)	122	134	129	127
Percentage using a vitamin A supplement	68%	41%	42%	35%
Percentage using oral contraceptives	10%	26%	21%	17%
MEN				
Number	24	13	13	50
Mean age	27.9	27.9	23.7	26.8
Mean height (inches)	71.2	70.4	71.1	70.9
Mean weight (pounds)	155	161	156	157
Percentage using a vitamin A supplement	33%	54%	46%	42%

V = Vegetarians
M-O = Moderate Omnivores
O = Omnivores

TABLE 6

MEAN DAILY DIETARY VITAMIN A CONSUMPTION (IU) ± STANDARD DEVIATION

	V	M-O	V	ALL
TOTAL VITAMIN A				
Women	10,377 ± 5900	11,622 ± 7296	10,433 ±9926	10,757
Men	12,237 ± 6641	8,189 ± 6096	9,187 ± 4466	10,392
PREFORMED VITAMIN A				
Women	2,082 ± 1491	2,135 ± 929	4,531 ± 7799	2,736
Men	2,292 ± 1804	4,622 ± 4855[a]	4,090 ± 2272[b]	3,365
PROVITAMIN A				
Women	8,295 ± 5546	9,487 ± 7103	5,902 ± 4645	8,023
Men	9,945 ± 6635[c,d]	3,567 ± 3489	5,097 ± 3251	7,025

a= significantly greater than V, $p < .05$
b= significantly greater than V, $p < .025$
c= significantly greater than M-O, $p < .005$
d= significantly greater than O, $p < .025$

	WOMEN	MEN
Vitamin A supplement users	10,799	9,295
Non-users	10,965	11,344

AGES	WOMEN n	WOMEN IU	MEN n	MEN IU
15-19	10	8871	5	8721
20-29	70	10,693	32	8781
30-39	10	9406	9	13,861[a]
40-45	2	29,196	4	17,564[b]

a= significantly greater than ages 20-29, $p < .025$
b= significantly greater than ages 20-29, $p < .005$

male M-O and O, who, in turn, ingested much more of the preformed vitamin. Men and women ate approximately the same amount of vitamin A each day, the difference being only 3.5%, though distribution between provitamin and preformed vitamin varied to a greater degree.

The women's mean daily consumption of 10,757 IU was about two and a half times both their RDA and the mean intake of comparable women in the HANES survey while the men's 10,392 IU exceeded their RDAs and the HANES sample by 60% to 139%. This surplus may be partly due to the fact that all the volunteers for this study were inherently concerned with their nutritional welfare as well as due to the availability in early autumn of fresh tomatoes, cantaloupes, corn, and other produce rich in vitamin A. Nevertheless, as shown in Table 7A on page 33, one of five men and one of seven women did not satisfy their RDA for vitamin A over the three-day experimental period.

The sources of dietary vitamin A for the 92 women and 50 men are detailed in Table 8 on page 34. Women consumed a significantly greater proportion from vegetables and a significantly smaller percentage from dairy products, potatoes, and corn. In general, they acquired 13% more of their vitamin A from carotenoids than did the men.

Tables 9 and 10 on pages 35 and 36 compare the diets of V, M-O, and O. For women, V and M-O derived anout three-quarters of their vitamin A from vegetables and fruits and, compared to the O, a smaller fraction from butter, margarine, sweets, meat, and fish. There were no important differences in the proportional consumption of fruit, dairy products, eggs, fortified cereals, potatoes, corn, fats, bakery goods, legumes, seeds, and nuts.

TABLE 7 A

PERCENTAGES OF SUBJECTS CONSUMING MORE THAN TWICE THEIR RECOMMENDED DIETARY ALLOWANCES AND LESS THAN THE RECOMMENDED DIETARY ALLOWANCES OF VITAMIN A DURING THE THREE-DAY DIET PERIOD.

	V	M-O	O	TOTAL
MORE THAN TWICE RDA				
Women	59%	63%	54%	59%
Men	42%	23%	31%	34%
LESS THAN THE RDA				
Women	10%	19%	17%	14%
Men	13%	46%	8%	20%
LESS THAN TWO-THIRDS THE RDA				
Women	2%	4%	0%	2%
Men	0%	8%	0%	2%

TABLE 7 B

PERCENTAGES OF SUBJECTS CONSUMING LESS THAN THEIR RECOMMENDED DIETARY ALLOWANCE OF VITAMIN A ON A 75% CAROTENOID DIET. SEE TEXT, PAGE 38.

	V	M-O	TOTAL
Women consuming less than 6280 IU	34%	33%	34%
Men consuming less than 7850 IU	29%	---	29%

TABLE 8

SOURCES OF DIETARY VITAMIN A: PERCENTAGES OF MEAN DAILY INTAKE FOR MEN AND WOMEN.

	WOMEN	MEN
Vegetables	59.0[a]	48.7
Fruit	11.6	11.5
Dairy products	11.4	17.7[b]
Eggs	4.9	4.3
Fortified products	4.0	6.0
Butter/margarine	3.5	3.1
Meat, fish	2.2	2.7
Sweets (pastry, ice cream, cookies, cake)	1.8	2.4
Potatoes, corn	0.7	1.9[c]
Bakery goods	0.6	0.5
Fats	0.5	0.6
Legumes,seeds,nuts	0.1	0.1
	100.3	99.5

a= significantly greater than men, $p < .01$
b= significantly greater than women, $p < .005$
c= significantly greater than women, $p < .05$

TABLE 9

SOURCES OF DIETARY VITAMIN A FOR WOMEN: PERCENTAGES OF MEAN DAILY INTAKE

	V	M-O	O
Vegetables	61.7[a]	64.8[a]	47.8
Fruit	12.2	10.1	12.2
Dairy foods	11.7	10.4	11.8
Eggs	5.3	5.5	3.5
Fortified products	3.0	4.0	5.5
Butter/margarine	2.7	2.8	6.0[b]
Sweets (pastry, ice cream, cookies, cake)	1.3	1.3	3.2[c]
Cereals, corn	0.9	0.7	1.0
Fats	0.4	0.3	1.0
Bakery goods	0.4	0.4	1.0
Meat, fish	0.3	0.5	7.5[c]
Legumes, seeds, nuts	0.1	0.1	0.0
	100.0	100.9	100.5

a= significantly greater than O, $p < .01$
b= significantly greater than V, M-O, $p < .01$
c= significantly greater than V, M-O, $p < .025$

TABLE 10

SOURCES OF VITAMIN A FOR MEN: PERCENTAGES OF MEAN DAILY INTAKE.

	V	M-O	O
Vegetables	59.3[a]	34.8	43.1
Fruit	14.8	8.5	8.3
Dairy products	13.0	22.5	21.7
Butter/margarine	4.0	4.2	3.8
Eggs	3.0	6.2	4.9
Fortified products	2.5	10.1	8.3
Cereals, corn	2.2	2.8	0.6
Sweets (pastry, ice cream, cookies, cake)	0.7	3.2[b]	4.8[c]
Bakery goods	0.4	0.2	1.2
Fats	0.4	1.0	0.7
Meat, fish	0.3	6.3	2.7
legumes, seeds, nuts	0.1	0.1	0.0
	100.7	99.9	100.1

a= significantly greater than M-O, $p < .01$
b= significantly greater than V, $p < .025$
c= significantly greater than V, $p < .01$

TABLE 11

PERCENTAGE OF MEAN DAILY VITAMIN A INTAKE DERIVED FROM PROVITAMINS AND FROM PRE-FORMED VITAMIN A.

	V	M-O	O	TOTAL
PROVITAMIN				
Women	74.9	75.7	61.0	71.4
Men	76.4	46.2	52.0	62.2
PRE-FORMED VITAMIN				
Women	25.1	24.3	39.0	28.6
Men	23.6	53.8	48.0	37.8

TABLE 12

PERCENTAGE OF MEAN DAILY VITAMIN A INTAKE DERIVED FROM CARROTS.

	V	M-O	O	TOTAL
Women	39.4[a]	36.8[a]	18.8	33.3[b]
Men	30.5	13.9	15.8	22.4

a= significantly greater than O women, $p < .01$
b= significantly greater than men, $p < .025$

Among the men a sharper distinction emerged between V and M-O. The former preferred vegetables and fruits for three-quarters of their vitamin A while the latter acquired less than half that way and relied upon proprotionately more dairy foods, fortified cereals, meat, eggs, and sweets. (In contrast, female V and M-O were nearly indistinguishable.) Male M-O and O derived more retinol from sweets and less from vegetables than the V. In all other categories of food, the differences among the three groups were not statistically important.

The Food and Nutrition Board's estimation that half of American dietary vitamin A is provitamin proved to be reasonably accurate for male M-O and O, less so for female O, and not at all for the three remaining groups. As described in Table 11 on page 37, both V groups as well as female M-O secured about three-quarters of their vitamin A from plant sources. Since carotenoids are not absorbed as efficiently as the preformed vitamin (14), the effective dietary intake in these circumstances would tend to be overestimated. According to a formula developed by the World Health Organization for adjusting dietary requirements when the provitamin content is elevated, a 75% carotenoid diet would increase the daily requirement 57% over the level necessary with a 50% carotenoid diet (74). If this is realistic, then about one-third of all the V and women M-O in this study would not have satisfied their RDA during the three-day period (see Table 7B on page 33).

Some of the most striking differences in vitamin A intake centered on carrots, which hold about 11,000 IU per 100 grams. Table 12 on page 37 shows that women V and M-O reaped about 37% to 39% of their total vitamin A from carrots alone, about twice the amount as O, and male V about 30%, twice that of M-O and O. Women overall consumed a substantially greater quantity of carrots than the men.

Mean plasma vitamin concentrations for 80 women and 40 men are shown in Table 13 on page 40. As expected (13), women had lower levels than men, about 17%, and oral contraceptive users higher levels than non-users, about 37%. Both male and female M-O enjoyed slightly higher concentrations than the others of their sex, but differences among dietary groups were not statistically significant. This held true for vitamin A supplement users as well as for different age groups.

The laboratory means of 54.1 ug% for men and 46.3 ug% for women agree quite well with other biochemical surveys. The HANES study found 62.1 ug% for white males and 53.0 ug% for white females aged 18-44 years (30). Twelve hundred British subjects examined from 1948-1957 registered mean blood levels of 52 ug% for men and 43 ug% for women (48). The Ten-State Nutrition Survey of 1968-1970 reported a mean of 49.1 ug% and 44.3 ug% for men and women, respectively, residing in low income states (49). None of these surveys used a fluorometric method.

None of the women or men possessed plasma vitamin A concentrations which fell below 20 ug%, the threshold most often regarded as distinguishing acceptable from unacceptable levels (75). Others place it at 30 ug% (13), a cut-off that 9% of the women but none of the men in this study would have failed. In a comparable sample from the HANES survey, 2.5% of the women and 1.3% of the men were found with levels less than 30 ug% (30). Suspicion about the accuracy of the spectrofluorometric method, especially in assaying women's plasma, precludes drawing any firm conclusion about the prevalence of low vitamin A concentrations in this pop-

TABLE 13

MEAN PLASMA VITAMIN A CONCENTRATIONS (ug/ 100 ml ± S.D.)

	n	V	M-O	O	ALL
Women	80	46.0 ± 11.6	49.0 ± 11.8	44.1 ± 16.7	46.3 ± 13.1
Men	40	51.9 ± 11.2	59.8 ± 18.7	53.6 ± 11.1	54.1 ± 13.2

	WOMEN	MEN
Vitamin A supplement users	46.3	56.2
Non-users	46.4	52.8
Oral contraceptive users	59.7[a]	
Non-users	43.7	

a = significantly greater than non-users, $p < .01$

	WOMEN		MEN	
AGES	n	Vit.A	n	Vit.A
18-19	8	49.7	3	41.0
20-29	62	46.4	25	55.0
30-39	9	43.7	9	55.6
40-45	1	35.4	3	54.3

ulation until the blood values can be verified by chromatography or another assay method.

In general, there was no correlation between the level of vitamin A circulating in the blood and the total vitamin A ingested; the correlation coefficient, r, for males was only 0.04 and for females a mere 0.02.

The 40 males were divided into quartiles and the 67 women not using oral contraceptives into quintiles according to vitamin intake in order to detect any relationships between the dietary levels of preformed vitamin A, provitamin A, or the ratio between these two, and the level of the vitamin in the plasma. A few significant correlations were ntoiced but no clear trend appeared. See Tables 14 and 15 on pages 42 and 43. Males consuming the least amounts of preformed vitamin A and the highest proportions of provitamins tended to have lower concentrations of plasma vitamin A, but this was not true for females. Those, male and female, who ingested the greatest quantity and highest proportion of preformed vitamin did not exhibit an elevated plasma level of the vitamin.

In summary, it appears from these results that among an educated, young, financially comfortable population meat eating habits do not have a notable impact upon vitamin A status. Despite a diversity of food sources, total intakes and blood concentrations of vitamin A remain similar. This confirms some of the work of Hardinge and Stare from twenty-five years ago (38).

Still, the participants in this study may have been too homogeneous,

TABLE 14

CORRELATIONS BETWEEN VITAMIN A INTAKE AND PLASMA CONCENTRATION IN MALE SUBJECTS DIVIDED INTO QUARTILES ACCORDING TO INTAKE.

IU	n	MEAN IU	PLASMA ug%	r
		TOTAL VITAMIN A INTAKE		
16,808-26,202	10	19,722	52.4	.01
9,446-16,802	10	11,223	55.3	.33
5,481- 9,286	10	7,578	52.2	.37
2,045- 5,988	10	4,359	56.3	.58
		PRE-FORMED VITAMIN A INTAKE		
4,033- 8,769	10	6,334	56.2	.17
3,439- 3,916	10	3,632	57.7	.26
1,698- 3,436	10	2,326	56.2	.17
0- 1,610	10	848	46.0	.70[a]
		PROVITAMIN A INTAKE		
10,637-24,158	10	16,470	53.7	.12
6,143-10,001	10	7,748	49.5	.32
2,256- 5,895	10	4,028	57.0	.19
319- 2,244	10	1,495	56.0	.17

	RATIO OF PREFORMED VITAMIN TO PROVITAMIN INTAKE			
RATIO	n	MEAN RATIO	PLASMA ug%	r
1.129-14.166	10	3.762	57.3	.29
0.602- 1.092	10	0.796	53.7	.37
0.257- 0.599	10	0.423	57.3	.27
0.000- 0.249	10	0.091	47.9	.71[a]

a = $p < .05$

TABLE 15

CORRELATIONS BETWEEN VITAMIN A INTAKE AND PLASMA CONCENTRATION IN FEMALE SUBJECTS DIVIDED INTO QUINTILES ACCORDING TO INTAKE.

IU	n	MEAN IU	PLASMA ug%	r
		TOTAL VITAMIN A INTAKE		
14,426-50,962	13	23,822	44.7	.03
12,101-14,369	13	12,925	44.9	.23
9,065-11,715	13	10,065	41.6	.21
5,664- 8,973	14	6,731	37.0	.29
1,332- 5,611	14	4,216	50.5	.18
		PRE-FORMED VITAMIN A INTAKE		
3,087-38,725	13	7,271	41.4	.31
2,293- 2,994	13	2,656	42.1	.39
1,706- 2,198	13	1,907	42.6	.04
1,286- 1,699	14	1,516	48.2	.09
500- 1,257	14	951	44.0	.11
		PROVITAMIN A INTAKE		
11,103-27,188	13	17,724	46.8	.50
9,603-11,042	13	11,[illegible]09	44.7	.25
6,404-8,842	13	7,[illegible]78	39.2	.19
2,205- 6,377	14	4,368	43.7	.35
629- 1,403	14	2,515	44.2	.57[a]

RATIO	n	MEAN RATIO	PLASMA ug%	r
		RATIO OF PRE-FORMED VITAMIN TO PROVITAMIN INTAKE		
0.668-3.165	13	1.381	41.3	.38
0.410-0.644	13	0.519	42.5	.30
0.229-0.367	13	0.308	48.5	.38
0.143-0.222	14	0.188	42.6	.15
0.032-0.134	14	0.089	43.9	.34

a = $p < .05$

autumn too tempting with cantaloupes, tomatoes, and other produce rich in vitamin A, and the vegetarians too green and unseasoned for pronounced contrasts to have emerged. Perhaps with vegetarians of longer standing and with omnivores less attentive to their diet the outcome would have been different.

Several details which surfaced in the study merit additional research. If the diets of the vegetarians and the moderate omnivore women had been assessed by the World Health Organization, about one-third would have been judged deficient in vitamin A (see page 38), while if judged by the Food and Nutrition Board's RDA guideline less than a fifth would have been. Should the U.S. RDA which assumes a 50% provitamin A content be modified for those consuming higher percentages of provitamins? Is the absorption of carotenoids, especially the carotene in carrots, so inferior to the preformed vitamin that there is cause for alarm in some American diets?

Women using oral contraceptives once again showed considerably higher plasma concentrations of vitamin A. Does this phenomenon result in its increased catabolism? Should the dietary requirement be boosted for these women?

Curiously, women taking vitamin A supplements had virtually identical dietary intakes and blood levels of the vitamin as those who didn't. What impact does occasional or regular ingestion of synthetic vitamins really have upon nutritional status? What types of people take these supplements, for what reasons, and what effect does this have on their dietary habits? For instance, do they subsequently become complacent about their food choices and cooking methods?

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