THE AVAILABILITY TO THE RAT OF CERTAIN CAROTENES IN RAW AND COOKED VEGETABLES

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THE AVAILABILITY TO THE RAT OF CERTAIN CAROTENES IN RAW AND COOKED VEGETABLES

The literature presents many discrepancies concerning the utilization of carotene as vitamin A. Guggenheim (1), Fraps and Meinke (2) and Treichler, Kemmerer and Fraps (3) have all reported that the carotene in different foods is not as well utilized by the rat for storage of vitamin A in the liver as is carotene in cottonseed oil. Many theories have been advanced as to the probable causes but the work of Kemmerer and Fraps (4) has substantiated that foods which contain similar total amounts but varying proportions of different carotenes have different biological values.

Human foods have not been studied extensively as to their relative proportions of the biologically active carotenes and very little has been done on the effect of cooking on these. Therefore a study of the effect of home cooking of spinach and carrots on the concentration of alpha- and beta-carotene and the biological values of these was undertaken.

This research is reported under the following headings:

1. The alpha- and beta-carotene content of raw, cooked and frozen stored spinach and carrots.
2. The relative values of alpha- and beta-carotene, vitamin A alcohol, raw and cooked spinach, and carrots for growth of the rat.
3. The fecal excretion and absorption of various carotenes and storage as vitamin A in the liver of the rat.

1. THE ALPHA- AND BETA-CAROTENE OF RAW, COOKED AND FROZEN SPINACH AND CARROTS

Both carrots and spinach or other leafy vegetables may contribute a large proportion of carotene to the human diet. This has been especially true during the war and post war years when the animal sources of vitamin A have been scarce. Considerable discrepancy has been reported in the literature in the carotenoid content of vegetables, as determined by chemical and physical methods, and the actual potencies found by animal feeding experiments. It appeared possible that, in some cases, the analytical methods might be unreliable in that non-active carotenoids were determined and calculated as provitamin A - active pigments. Kemmerer and co-workers (4,5) investigated the constituents of the crude carotene of some human foods. These investigators, using chromatographic methods, found that 76 per cent of the crude carotene from leafy vegetables was in the form of beta-carotene, while raw carrots contained 62 per cent of the total carotene in the beta form. They have shown that the carotene extracts of plants are complex in nature and may consist of beta-carotene, of impurity A, of neo-beta-carotenes B and U and alpha-carotenes. The neo-beta carotenes are stereoisomers of beta-carotene and can be formed from and converted to beta-carotene. The crude carotene content of fresh carrot was found to range from 386 to 1120 p.p.m. Zscheile and co-workers (6,7) reported that spinach contained 60 micrograms per gram total carotene of which 87 to 89 per cent was beta-carotene and 11 to 13 per cent neo-beta-carotene. Cooking for ten minutes showed an increase in total carotene but no change in the percentage beta-carotene. During frozen storage raw spinach lost considerable of its total carotene and its percentage of beta-carotene was reduced. Fraps and Meinke (2) found that spinach contained

61 p.p.m. total carotene of which 52 per cent was beta-carotene, 14 per cent neo-beta-carotene B, 17 per cent neo-beta-carotene U and 17 per cent impurity A.

In an investigation of the carotenoid content of 16 varieties and 18 strains of carrots, Harper and Zscheile (8) used both chromatographic and spectrographic means of analysis. Garden varieties of carrots were found to average 54 micrograms of carotene per gram of material, of which 46 per cent was alpha-carotene and 54 per cent was beta-carotene; zeta-carotene appeared rather generally and lycopene was present in certain varieties. These results indicate a lower beta-carotene concentration than is generally assumed. Kemmerer and Fraps (4, 9) have reported values of 52 to 65 per cent beta-carotene and have accounted for 94 per cent of the total carotenes as alpha- and beta-carotenes separated chromatographically.

Harper and Zscheile's findings of 46 per cent alpha-carotene is higher than the highest value of 36 per cent found by Mackinney (10) in a survey of leaf carotene. Harper and Zscheile (8) consider that the high percentage of alpha-carotene found in carrots is an important factor in the estimation of provitamin A activity as it could cause errors of 25 per cent in the biological potency. However the other carotene constituents are less than 10 per cent of the total carotenes thus could only influence vitamin A activity slightly.

The literature contains little on the effect of cooking or frozen storage on carrots and spinach thus a study of the effect of these on the total carotene content and the percentage alpha- and beta-carotene was undertaken.

EXPERIMENTAL PROCEDURES:

Processing and Sampling:

Approximately 10 pounds of spinach, purchased on the local market in May and November 1945 was washed, midribs removed, cut into small pieces and mixed. Lots of 500 grams were cooked for 10 minutes in 2.5 times the weight of boiling water. The drained cooked weights were obtained. Both the raw and cooked spinach, in 10 gram lots were packaged and sealed in cellophane bags and quick frozen at -40°F. and stored at -10°F. Carrots purchased on the local market, at the same time, were washed, cut in half longitudinally, using one-half for the raw sample and the other for the cooked. They were then grated crosswise. The cooking sample was cooked for nine minutes in 2.5 times its weight of boiling water. Both the raw and cooked samples were packaged and stored in the same manner as the spinach. This procedure of preparation was used to minimize the sampling error and to secure uniform sample for animal feeding.

The vegetables were analyzed spectrographically for carotene on the same day they were processed. This procedure was repeated at later storage periods.

Chemical Methods:

Samples of approximately five grams were extracted with absolute ethyl alcohol in a Waring blendor (a small amount of calcium carbonate was added to counteract any effects of plant acids which might be liberated). The suspension was filtered on a Buechner funnel and the residue washed repeatedly with petroleum ether until all pigment was removed. The filtrate was transfered to a separatory funnel, adjusted to 85 per cent alcohol with water, and the carotene extracted with petroleum ether. The solution was

washed with 90 per cent methyl alcohol, and the chlorophylls removed by shaking for one minute with 20 per cent potassium hydroxide in methanol (7). The petroleum ether extract was washed free of alkali, dried over sodium sulphate, evaporated under reduced pressure and the residue dissolved in cyclohexane (Eastman's) and made to volume. Spectrographic analyses were made on a Beckman quartz photoelectric spectrophotometer.

Absorption values were determined at wave lengths 4340, 4530, 4700 and 4820 A^{o}, which are co-incident points of the curves for alpha- and beta-carotene in cyclohexane. Specific absorption coefficients at these wave lengths for cyclohexane solutions are 159, 215, 175 and 186 liters per gram centimeter, respectively. Wave length 4530 A^{o} was chosen for superposition of the characteristic curves of the vegetable extract as Harper and Zscheile (8) had found this point satisfactory for carrot extracts. Absorption at wave length 4820 A^{o} was used as an index of isomerization. Wave lengths 4820 and 4900 A^{o} were used for analyses of extracts as two-component systems for alpha- and beta-carotene according to the method of Beadle and Zscheile (7). The ratio of wave lengths given in Table 2 refer to ratios of relative absorption value at two indicated wave lengths.

The standard absorption curves and coefficients were determined on alpha- and beta-carotene (General Biochemicals, Inc.) in cyclohexane purified for spectrographic determinations (Eastman, Inc.) and shown in Figure 1 and Table 1.

RESULTS AND DISCUSSION:

Most carotene research has been conducted using hexane as a solvent but cyclohexane is equally as satisfactory from wave length 3000 to 5000 A^{o} and was more available in 1945. Therefore it was chosen in this research

for both the carotene and vitamin A determinations. Table 1 presents the specific absorption coefficients used in this study for alpha- and beta-carotene at the coincident points and the widely separated points of the characteristic curves. The absorption curves are presented in Figure 1 with the curves for raw spinach.

The only references to beta-carotene in cyclohexane are given by Devine et al (11) and Braude and co-workers (12). They report a maximum at 4560 A° with specific absorption coefficients of 249 and 240 respectively. In this study the maximum was at 4530 A° with an absorption coefficient of 215. An earlier sample of beta-carotene (General Biochemicals, Inc.) had absorption coefficients which agree better with the previous work with an absorption value of 240 at the maximum point 4530 A°.

Differences in the sample lots purchased were also noted for alpha-carotene. The sample used in this research had a maximum at 4490 A° with an absorption coefficient of 227 l. per gm. cm. while the earlier purchase had the same maximum point but an absorption coefficient of 357. Since there was insufficient of the first lot for repetition of results or the continuance of the experiment the results from the second lot have been used as standards throughout this research.

The carotene contents of the raw and cooked spinach and carrots during various periods of frozen storage are reported in Table 2. The effects of storage on the characteristic curves as compared to the initial product and to alpha- and beta-carotene are presented for raw and cooked spinach and raw and cooked carrot in Figure 1, 2, 3 and 4 respectively.

Spinach purchased in May contained 4.19 mg. of total carotene per 100 g., November spinach 5.96 mg. and the percentage beta-carotene was 76.4 and 81.6 respectively. When cooked, May spinach contained 6.33 mg. total carotene

per 100 g., November spinach 6.92 mg., in which the beta-carotene content was 86.5 and 94.1 per cent respectively. Carrots purchased in May contained 14.00 mg. of total carotene per 100 g. whereas the November sample contained 10.53 mg. The percentage beta-carotene was 66.2 and 76.4 respectively. When cooked, the total carotene concentration of the May sample was 12.79 mg. and the November sample 9.64 with a beta-carotene concentration of 58.6 and 62.3 per cent respectively.

The total concentrations in Table 2 do not check as determined at the various coincident points. This is in agreement with the results of Harper and Zscheile (8) and Kemmerer (13). In every case, the results calculated at 4340 A^{o} are higher than at the wave lengths 4530, 4700 or 4820 A^{o}. This undoubtedly is because the carotene extracts contained not only alpha- and beta-carotene but also others which Harper and Zscheile (8) identified as zeta-carotene in carrot extracts. Kemmerer, (13) and Harper and Zscheile, (8) have reported that the results obtained at 4500 A^{o} in hexane which compares to 4530 A^{o} in cyclohexane are very satisfactory for total carotenes although the latter workers state that 4782 A^{o} (4820 A^{o} in cyclohexane) is the most reliable for the content of the combined alpha- and beta-carotenes. This latter point was used by Strain (14) in the analysis of butterfat carotenoids. Wave length 4900 A^{o}, according to Harper and Zscheile, (8) is satisfactory for estimating alpha- and beta-carotene contents individually, although the presence of gamma-carotene or lycopene will cause such results to be high in favor of beta-carotene.

Table 2 shows that the cooking of spinach increases the total carotene content 45.5 per cent for sample 1 and 23.1 for sample 2. However, when these were calculated on the total weight of the raw spinach used in cooking and the drained weight of the cooked, then these gains became 10.1 and 1.2 per cent respectively, indicating that these changes were due to the

inaccuracies involved in obtaining a comparative weight of the drained products. Cooking of carrots produced losses of 2.1 and 15.1 per cent respectively but when calculated on the total amounts cooked then the losses were 11.0 and 7.8 per cent.

The percentage of beta-carotene (Table 2) appears to be greater in the cooked than in the raw spinach. This may be because the absorption at wave lengths 4820 to 5000 A^{o}, Figures 1 and 2, is greater than that of raw spinach, becoming almost identical with the beta-carotene curve. Harper and Zscheile, (8) reported that ratios of absorption values, chosen from the standard curves of pigments known or thought to be present in the extract are very helpful as an indication of pigment identity. Wide departures of ratios from expected values indicates the presence of additional pigments. In this work, the ratios $\frac{4780}{4820}$ and $\frac{4900}{4820}$ were chosen and these ratios for pure alpha- and beta-carotene ranged from 1.070 to 0.989 and 0.584 to 0.843 respectively. All the values in Table 2 were in this range for the ratio $\frac{4900}{4820}$, except both samples of cooked spinach which remained consistently high throughout storage. This may indicate the presence of some isomer which has a greater absorption than beta-carotene at wave length 4900 A^{o}. This change in the characteristic absorption curve of cooked spinach may explain the apparent increase in percentage beta-carotene.

Frozen storage of raw spinach caused losses in total carotene content of 6.7 per cent in three months, 49.2 in 8 months, 43.5 in 10 months and 59.4 in 15 months. Zscheile, Beadle and Kraybill (6) reported that raw spinach (Giant Noble) lost 63.5 per cent of total carotene in 11 months and (King of Denmark) 57.1 per cent in 11 months and 51.7 in 12 months. These losses were considerably greater than what was reported for 10 months here but in this case the variety was not known and the spinach purchased in

May was probably of a different variety than that bought in November. The character of the absorption curve, Figure 1, was markedly changed after 10 months storage. In general, the absorption coefficients were increased above those for raw spinach from 4000 to 4530 A° and from 4560 to 4760 A° and below it from 4760 to 5000 A°. This indicates isomerization and the general shape of the curve resembles that of Zechmeister (15) for beta-carotene after iodine treatment and that of Zscheile and Porter (16) for neo-beta-carotene.

Cooked spinach, on storage, showed an apparent gain in total carotenes but it is questionable whether this is an actual gain or whether it is due to the change in the character of the absorption curve. These results do not compare to the losses of 26.4 per cent in 11 months reported by Zscheile and co-workers (6) for blanched spinach (Giant Nobel). This may be because cooking for nine minutes in boiling water destroys more enzymes than blanching for two minutes. The changes in the specific absorption curve due to storage (Figure 2) resemble those of the curve for raw spinach stored 10 months below 4820 A° except that they are less marked, and above this wave length it is identical with the freshly cooked product.

Carrots purchased in May contained more total carotene than those bought in November but the percentage beta-carotene was less. Both the total carotene and the percentage beta-carotene are in the range reported by Harper and Zscheile (8) and Kemmerer and Fraps (9) using a chromatographic method.

The shape of the absorption curve of cooked carrot resembles that for raw carrot but its absorption is greater from 4000 to 4420 A° and less from 4770 to 5000 A°. The percentage beta-carotene is less in the cooked product than in the raw.

Raw carrot during frozen storage showed no changes in total carotene in three months but losses of 26.4 per cent in eight months, 14.7 per cent in 10 months and 44.1 per cent in 15 months. There was no marked change in the percentage beta-carotene. The absorption curve of the stored raw carrot, (Figure 3) did not show the marked change produced in raw spinach. It was lower than the fresh product from wave length 4000 to 4340 A°, practically identical from 4420 to 4630 A°, higher to 4770 A°, lower than the fresh to 4860 A° and then identical to 5000 A°.

Cooked carrot, during frozen storage, showed no significant change in total carotene. The percentage beta-carotene as measured spectrographically, was increased slightly. The absorption curve for cooked carrots stored for 10 months, was similar to that of raw carrots, except slightly more exaggerated between 4650 and 4770 A°.

When the characteristic curves of the extracts of raw and cooked spinach and carrots (Figures 1, 2, 3, and 4), which were not subjected to frozen storage, are superimposed to agree at 4530 A°, they also agree well at 4700 and 4820 A° for spinach but the raw carrot is slightly higher than the cooked at 4820 A°. These wave lengths are coincident points for alpha- and beta-carotene. When the vegetables were stored 10 months, their coefficients were noticeably increased at 4700 A° which indicates the formation of some of the cis-forms of these pigments but which were not present in any great concentration in either the raw or the cooked vegetables. This also indicates that 4700 A° may not be a very accurate point for measuring total carotene in processed vegetables.

At wave lengths below 4400 A° the curves for the vegetable extracts usually are higher than the standard curves of either alpha- or beta-carotene especially from wave lengths 4250 to 4350 A° and 4000 to 4150 A°. This is in agreement with the work of Harper and Zscheile (8) on carrots in which they claim that this increase is due to a carotene, provisionally named zeta-carotene by Strain and Manning (17), which has major maxima in

the same position as those of the pigment designated as unnamed carotene 1 by White, Zscheile, and Brunson (18). It was prepared from carrots by them and also by Strain (14) and Strain and Manning (17).

Harper and Zscheile (8) reported that the carotenol fraction of most commercial carrots was a relatively small part of the total carotenoids, approximately 10 per cent and that cryptoxanthol may form part of this. The alpha-carotene fraction as determined in the work reported here may contain some cryptoxanthol. However, any error in calculation owing to the analyses of cryptoxanthol as alpha-carotene would be insignificant from the biological standpoint because of the similarity in provitamin A potency and the small quantities involved.

SUMMARY AND CONCLUSIONS:

Spinach and carrots, purchased on the local market in May and November were analyzed spectrographically for total carotenes, alpha- and beta-carotene in the raw and cooked states and at various periods after frozen storage.

Spinach purchased in May contained 4.19 mg. of total carotene per 100 g., November spinach 5.96 mg. and the percentage beta-carotene was 76.4 and 81.6 respectively. Carrots grown in May contained 14.00 mg. of total carotene per 100 g. and the November sample 10.53 mg. and the percentage beta-carotene was 66.2 and 76.4 respectively.

Frozen storage caused little change in the total carotene content of either raw spinach or carrots in three months but caused losses up to approximately 50 per cent by 15 months storage. There was little change in the total carotenes of the cooked products.

Absorption curves are presented for alpha- and beta-carotene in cyclohexane and for extracts of the raw and cooked spinach and carrots immediately after purchase and after 10 months frozen storage. Cooking produced a greater change from the raw product in the absorption curve of carrots than

of spinach. Frozen storage of raw spinach and carrots caused a marked change in the absorption curves, indicating isomerization. The changes in the cooked products were less significant, indicating that this process protected their carotene content.

2. THE RELATIVE VALUE OF ALPHA- AND BETA-CAROTENE, VITAMIN A ALCOHOL, RAW AND COOKED SPINACH AND CARROTS FOR GROWTH OF THE RAT

Considerable attention has been given to the discrepancies between chemical determinations of carotene and the results of bioassay methods. Much effort has been expended in trying to discover the cause or causes of this apparent discrepancy.

Lease and co-workers (19) and Sherman (20, 21) have observed that the utilization of carotene by the rat in curative growth tests is partially dependent upon the provitamin carrier. Smith and Otis, (22) reported that the quantity of vitamin A in the livers of rats fed carotene, after a depletion period, varied with the source. Using essentially the same technic, Guggenheim (1) has demonstrated that the utilization of carotene derived from various plant materials ranged from 33 to 67 per cent of that noted when preformed vitamin A was taken. In the case of lettuce, however, carotene utilization was found to equal that of the preformed vitamin.

Kemmerer and Fraps (9, 23) explained the variation between the chemical and biological methods as due to the fact that the total carotenes did not consist entirely of beta-carotene but usually contained impurity A, neo-beta-carotenes U and B and sometimes alpha-carotenes, in addition to the beta-carotene. Neo-beta-carotene B (9) and alpha-carotene (24) have 50 per cent of the vitamin A potency of beta-carotene; neo-beta-carotene U 25 (25) to 33 per cent (26) and impurity A (4) no apparent vitamin A

potency. Using rats as their test animal, Deuel and co-workers (27) reinvestigated the relative provitamin A potencies of cryptoxanthin and beta-carotene and in two independent assays obtained an actual pro-vitamin ratio of 54: 100 and 59: 100.

A study of the relative value of carotene in twenty-seven vegetables and feeds which compared the capacity of each foodstuff to promote growth in rats was made by Kemmerer and Fraps (25). At a low level of feeding, sufficient for moderate growth, the vitamin A value of the beta-carotene equivalent in plant materials, except carrots, appeared to be equal to that of beta-carotene in cottonseed oil. At high levels of feeding, for storage of vitamin A in the liver, previous reports have shown that the beta-carotene in plants is not nearly as well utilized as beta-carotene in cottonseed oil. The beta-carotene equivalent was the amount of beta-carotene plus 25 per cent the neo-beta-carotene U plus 50 per cent the neo-beta-carotene B plus 50 per cent the alpha-carotene.

Oser and Melnick (28) compared the bioassay data with the colormetric data on several plant foods, including spinach and carrots. They conclude that, on the average 1 microgram of crude carotene (free from xanthophyll and lycopene) as determined colorimetrically was equal to 1 U.S.P. unit of vitamin A by bioassay when tocopherols were fed.

Studies during the past several years, Bacharach (29), Davies and Moore (30) and Harris and co-workers (31) have demonstrated that vitamin E improves the utilization of carotene by protecting it against oxidation, primarily in the intestinal tract. Karrer and Keller (33) and Guggenheim (1) found better provitamin A utilization in lettuce than other vegetables. Karrer and Keller (33) showed that lettuce had the greatest amount of vitamin E of any of the vegetables tested. Guggenheim (1) demonstrated

that supplementation of the basal carrot diet with tocopherol doubled the biological value of the carotene content. These findings suggest that this may be one cause of variation in the responses to carotene feedings.

This research reports the relative value of alpha- and beta-carotene, vitamin A alcohol and total carotenes from raw and cooked spinach and carrots for a four week growth period of weanling rats.

EXPERIMENTAL PROCEDURES:

Rats, 22 to 23 days of age, from a colony maintained on a low vitamin A and vitamin D ration, were housed individually in screen bottomed cages and fed U.S.P. XII vitamin A free ration ad libitum. The entire consumption of food for the 28 day experimental period was recorded. Supplements of vitamin A or carotene were fed twice weekly in series 1 and four times weekly in series 2. Weekly growth records were kept. The animals were sacrificed on the morning of the 29th day. Gross examinations for abscesses in the middle ear, base of the tongue, glands of the neck and for abnormalities of the lungs, bladder and kidney were made.

The procedures for uniform sampling, cooking and storing the spinach and carrots are described in the preceeding section. The vegetables were prepared for rat feeding by removing individual frozen packages, weighing 10 grams and grinding in a micro Waring blendor with a heavy hydrolyzed starch solution. The resulting heavy suspension was made to volume in stoppered graduates and fed orally to the rat by measuring the required dosages in one milliliter hypodermic syringes. Fresh samples were prepared for each day's feedings. The vegetables were fed on the basis of the total carotene content as determined spectroscopically.

Vitamin A alcohol (Distillation Products, Inc.) was weighed and made to volume in cyclohexane. It was analyzed spectroscopically for vitamin A

whenever supplements for rat feeding were prepared. The required volume of the cyclohexane solution was evaporated under reduced pressure and the vitamin A dissolved and made to volume in wesson oil which contained the desired dosage of mixed tocopherols.

Alpha- and beta-carotenes (General Biochemicals, Inc.) were weighed and made to volume in wesson oil to which had been added the required dosage of mixed tocopherols. The concentrated solutions were analyzed regularly and dilute solutions prepared for feeding.

Mixed tocopherols (Vegol, a concentrate in oil obtained from Distillation Products, Inc.) were dissolved in oil and fed in the required concentrations to the negative and vegetable groups. All oil supplements were held in refrigerator storage. All the oil supplements were prepared so that 0.3 ml. of oil was fed orally from a one milliliter hypodermic syringe, twice weekly to series 1 and four times weekly to series 2.

Series 1 consisted of the following groups (five male and five female rats in each group); negative control, beta-carotene fed on levels of 0.45, 0.70, 1.03, 1.36 and 2.0 micrograms per rat per day, vitamin A alcohol 0.46 micrograms daily, alpha-carotene 2.36 micrograms per day, raw spinach, cooked spinach, raw carrot and cooked carrot in amounts supplying 3.82 micrograms daily of total carotenes as determined spectrographically. Series 2 consisted of the following groups of 10 animals each (five males and five females); negative control, beta-carotene 7.0 micrograms daily, alpha-carotene 23.6 micrograms per day, vitamin A alcohol 4.60 micrograms daily, raw carrot and cooked carrot in amounts supplying 20.0 micrograms of total carotenes per day. All groups in series 1 received 0.5 mg. of mixed tocopherols and in series 2, 1.0 mg. daily.

The growth data were corrected for the effects of initial weight and food consumption by analyses of covariance. The significance of gains between groups was determined by "t" values.

RESULTS AND DISCUSSION:

In this experiment the depletion period was omitted because, in the preceeding year, with the U.S.P. vitamin A method over 20 per cent of the rats died during the experimental period. The growth response of the remaining rats varied greatly. Weanling rats from a colony maintained on a low vitamin A ration, which permitted little storage of this vitamin, as indicated by an average depletion period of 21 to 22 days for males and 22 to 23 days for females, responded more uniformly to vitamin A than did depleted rats.

Table 3 presents the total carotene content and the percentage alpha- and beta-carotene of the various vegetables fed. These data were taken from the preceeding section. Chemical analyses showed that there was no significant variation during the storage period required for the bioassay.

The relationship of gain in weight to the daily supplements of beta-carotene is presented in Figure 5 for male and female rats, fed 0.45, 0.70, 1.03, 1.36 and 2.00 micrograms per rat per day. The group receiving 7.0 micrograms per rat per day was not included in the regression data since the growth response from this greater amount of carotene did not show a linear relationship to the lower levels fed. For the regression equation $y = a + bx$, the regression coefficient b was obtained from the formula $b = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$ and a from the formula $a = My - b\,Mx$ where y equals g. increase in weight of rats in four weeks and x equals the daily dose of beta-carotene in micrograms. The error of estimate was 2.34 g.

for the males and 1.43 g. for the females. In the males, three of the five groups were within one error of estimate from the regression line; in the females, four of the five were within one error of estimate. These regression lines were used for interpretating the results of the other supplements in terms of beta-carotene.

When the relationship of gain in weight to the daily supplement of beta-carotene was calculated by the method of Coward (34) using the logarithm of the daily dose practically identical results were obtained.

Table 4 presents the gain in weight, food consumption data, and the gain in weight adjusted by covariance for initial weight and food intake for the rats in series 1 and 2. Figure 6 shows graphically the relationship of the averaged adjusted gains in weight for males and females to the various supplements fed in series 1 and 2.

Table 5 presents the final covariance table of gain in weight, initial weight and food consumption for the combined groups in series 1 and 2. It is noted that both sex and treatment produced highly significant effects on the growth of the rats.

In series 1 all animals receiving carotene or vitamin A made significantly greater gains than the negative animals but there were no significant differences among groups receiving 0.45 micrograms, 0.70 or 1.03 micrograms beta-carotene daily. There were however significant differences in the gains of rats receiving 0.45, 1.36 and 2.00 micrograms of beta-carotene daily. These differences became progressively greater with greater differences in carotene intake. The results from these different feedings of beta-carotene produce the regression growth line (Figure 5).

When the growth of males and females were averaged together for groups fed 0.70 micrograms beta-carotene, 2.36 micrograms alpha-carotene and 0.46

micrograms and vitamin A alcohol, the gain in weight in each case was 98 g. in 28 days. On the same basis there was no significant difference in growth between groups of rats fed raw spinach, cooked spinach or cooked carrot. However, those fed raw spinach gained significantly more than those on raw carrot. (P.05)

When beta-carotene was fed at the levels of 0.45, 0.70 and 1.03 micrograms per day, there was no significant difference in the gains of males and females, but at all higher levels of feeding the differences were significant. The rate of growth, as shown by the slope of the regression line (Figure 5), was greater for males than females and this difference became significant when the carotene intake was greater than 1.03 micrograms per rat per day. The literature supplies very little information regarding the requirement of vitamin A of the female rat for growth.

When the other supplements are calculated as micrograms of beta-carotene on the basis of growth promoting abilities then the ratio of beta-carotene to vitamin A alcohol, alpha-carotene, the total carotenes of raw spinach, cooked spinach, raw carrot and cooked carrot for male rats are 1.00: 0.45: 2.03: 2.08: 2.25: 3.18: 2.56 respectively and for female rats 1.00: 0.72: 5.90: 3.29: 3.64: 6.47: 3.29 micrograms of beta-carotene respectively.

The estimated vitamin equivalent of beta-carotene to vitamin A alcohol of 1.00: 0.45 for male rats compares well to that of Harris and co-workers (31, 32), however he presents no data for female rats. The alpha-carotene ratio for male rats of 1.00: 2.03 compares favorably to data presented by Deuel et al, (24). The females however utilized the alpha-carotene only half as well for growth. The beta-carotene equiva-

lent of all vegetables was considerably lower than that reported by Oser and Melnick (28). By the chemical method employed the beta-carotene concentration of the cooked spinach was considerably greater than in the raw product. The bioassay does not substantiate this. It is possible that the cooking produced some unidentified isomers which were not measured by the spectrographic method used. Raw carrots had a lower beta-carotene content and their bioassay value was lower than the raw spinach. Cooked carrots had a higher biological value but not significantly so, than the raw although their beta-carotene content was lower. In this work any physical effect of the cooking of the vegetables on their digestibility was removed as all vegetables were ground to a fine suspension in a Waring blendor.

In series 2 all animals made significantly greater gains than those fed the lower concentrations of the same supplement in series 1. In this series there was no significant difference between the gains made on beta-carotene, alpha-carotene, raw carrot and cooked carrot at the levels fed. In the case of vitamin A alcohol, significantly poorer growth was obtained indicating that at higher levels of intake in series 2 growth was not stimulated to the same extent as it was when alpha- and beta-carotene were increased in the same proportions over that fed in series 1.

The negative group of rats fed 1.0 mg. daily of mixed tocopherols made significantly greater gains than those fed 0.5 micrograms in series 1, when the gains were corrected for initial weight and food intake. However, the food intake in series 1 was greater than in series 2, in fact, so much so that both groups were receiving essentially the same amount of vitamin E, supplied by the wesson oil (0.04% tocopherols). Since the ration, itself, contains this nutrient the difference shown by

the adjusted weights are unreliable for gains due to tocopherol intake. In series 1 the depletion period averaged 21 days for males and 22 for females, in series 2, 22 days for males and 23 for females.

SUMMARY AND CONCLUSIONS:

Weanling rats, from a colony maintained on a low vitamin A and vitamin D ration were fed U.S.P. XII vitamin A free ration ad libitum for 28 days. The first series consisted of negative control, beta-carotene fed at 0.45, 0.70, 1.03, 1.36 and 2.00 micrograms per rat per day, vitamin A alcohol 0.46 micrograms per day, alpha-carotene 2.36 micrograms per day, raw spinach, cooked spinach, raw carrot and cooked carrot in amounts supplying 3.82 micrograms daily of total carotenes as determined chemically. The second series consisted of the following groups, negative control, beta-carotene 7.0 micrograms daily, alpha-carotene 23.6 micrograms per day, vitamin A alcohol 4.6 micrograms daily, raw carrot and cooked carrot in amounts supplying 20.0 micrograms of total carotenes per day. The growth data were corrected for the effects of initial weight and food consumption by analyses of covariance.

A regression growth - dosage curve is presented for the lowest five levels of beta-carotene fed.

When the other sources of vitamin A were calculated to micrograms of beta-carotene from the growth response the ratio of beta-carotene to vitamin A alcohol, alpha-carotene, the total carotenes of raw spinach, cooked spinach, raw carrot and cooked carrot for male rats are 1.00: 0.45: 2.03: 2.08: 2.25: 3.18: 2.56 respectively and for female rats 1.00: 0.72: 5.90: 3.29: 3.64: 6.47: 3.29 micrograms of beta-carotene respectively.

Female rats did not utilize vitamin A alcohol, alpha-carotene or the carotenes from any of the vegetables as well as did the males for growth.

At the lowest levels of feeding there was no significant difference in the growth of males and females but at all higher levels there was a significant difference.

The total carotenes from spinach gave a better growth response than those from carrot, however, this difference was not significant. By chemical analyses spinach contained more beta-carotene than carrots. Cooking of the vegetables produced no significant difference in the bio-assay.

At the higher levels of feeding, in series 2, there was no significant difference between the gains made on 7.0 micrograms beta-carotene per rat per day, 23.6 micrograms alpha-carotene or 20.0 micrograms total carotenes from raw or cooked spinach but vitamin A alcohol fed at 4.60 micrograms per rat per day gave significantly poorer growth although it had compared favorably when the supplements were fed at a lower level in the first series.

3. THE FECAL EXCRETION AND ABSORPTION OF VARIOUS CAROTENES AND STORAGE AS VITAMIN A IN THE LIVER OF THE RAT

Researches on the absorption of carotenes are limited in number and the results are conflicting. Kemmerer and Fraps (35), With (36), Russell and co-workers (37) and Wilson et al (38) have reported the presence in hen and rat feces of yellow pigments other than carotene which may have interfered with the determination of carotene in feces and explain some of the variation of results. Most workers including Wagner (39), and Morgan and Bentley (40) have found that carotenes and vitamin A are excreted in the feces and not in the urine except under abnormal conditions.

Kemmerer and Fraps (35) and Treichler et al (3) report that carotene in oil was more completely utilized by rats than carotene in alfalfa.

However Booher and Callison (41) found that the carotenes of vegetables were utilized better by humans than carotene in oil. The relative digestibility of various carotenes by adult and by weanling rats was studied by Fraps and Meinke (2, 42). For adult rats the digestibility of beta-carotene was 57 per cent, alpha-carotene 64, crude carotene of boiled carrot 8, boiled mustard greens 55 and raw carrot 49 per cent. The digestibility of neo-beta-carotene-B was practically the same as beta-carotene. The weanling rats utilized 52 per cent of the beta-carotene and 53 per cent of the alpha-carotene when fed 60 micrograms in an oil which contained 83 per cent beta-carotene, 16 per cent alpha-carotene and one per cent impurity A.

The literature contains many reports on the storage of vitamin A in the liver when generous or massive dosages of vitamin A or carotene are fed but there is little information on the liver storagewhen the provitamin is fed at levels just supporting growth. Little, Thomas and Sherman (43), Caldwell, Mac Leod and Sherman (44), Roher and Sherman (45) and Campbell et al (46) reported that rats receiving 3.0 I.U. of vitamin A per gram of dry food or 0.8 I.U. per calorie stored negligible amounts of the vitamin in the liver. The vitamin A was measured in these researches by the Carr-Price or spectrographic method or by bioassay using the single feeding technic of Sherman and Todhunter. These workers found that 3.0 I.U. of vitamin A per gram of food was adequate for normal growth, reproduction and lactation. Using the Carr-Price method for determining the vitamin A in liver, Lewis and co-workers (47) found no liver storage below 10 I.U. daily and only slight or doubtful storage at 25 I.U. per day.

Callison and Knowles (48) report that rats maintained till 275 or 365 days of age stored no measurable amount of vitamin A in the liver when they received less than 50 to 80 I.U. of vitamin A daily per kg. of body weight. This is about four times the minimum requirement of the rat. They suggest

that the daily requirement of the rat for vitamin A is really higher than 20 I.U. per kg. of body weight per day and that some considerable need of the body must be met in an apparently normal animal before liver storage occurs.

Fraps and Meinke (42) studied the relative biological values of the carotenes of six different foods by determining the amount of the vitamin stored in the livers of rats over a fourteen day period. These authors found that carotene in vegetables possessed low biological value while carotene in butter and beef liver possessed high potency.

In 1942 Treichler, Kemmerer and Fraps (3) found what they termed pseudo vitamin A in liver. The vitamin A content of the liver as measured spectrographically was found to increase significantly during the two weeks following weaning despite the fact that they were fed a vitamin A free ration. He found that 60 I.U. daily of vitamin A in cod liver oil was the most efficient for liver storage, carotene was 59 per cent as effective and carotene in alfalfa 21 per cent.

The following research reports the absorption by the rat of alpha- and beta-carotene and carotenes from raw and cooked spinach and carrots at low and moderate intake. At the highest levels of intake it was considered worthwhile to study the vitamin A stores of the liver.

EXPERIMENTAL PROCEDURES:

The digestibility of the various carotenes was studied on groups of rats fed beta-carotene on levels of 0.45, 0.70, 1.03, 1.36, 2.0 and 7.0 micrograms daily; alpha-carotene 2.36 and 23.6 micrograms; total carotenes of 3.82 micrograms daily from raw and cooked spinach and carrots and 20.0 micrograms from raw and cooked carrot as fed in the previous experiment. The liver storage of vitamin A was studied on the groups of rats fed beta-carotene 7.0 micrograms daily, alpha-carotene 23.6 micrograms, vitamin A

alcohol 4.6 micrograms and 20.0 micrograms of total carotenes from raw and cooked carrot.

The feces from each rat were collected daily into individual stoppered bottles, which contained sufficient ethanol to cover. The bottle and alcohol were weighed at the beginning of the experimental period and again after the last collection of feces thus determining the fecal weight by difference. The fecal specimens were refrigerated during the experimental period and until analyzed for carotene.

On the 29th day when the animals were sacrificed, the livers were removed, the excess blood absorbed on filter paper, weighed, sealed in cellophane bags, labelled, quick frozen and stored until analyzed.

Chemical Methods for Carotene and Vitamin A.

Both the feces and livers were extracted in a Waring blendor (micro sized container) with alcohol, transferred to a low actinic boiling flask, 4 mls. of 40 per cent potassium hydroxide added, then refluxed for 30 minutes (standard taper flasks and condensers were used). The solutions were cooled, filtered, transferred to separatory funnels and the water, alcohol ratio adjusted. The solutions were extracted three times with petroleum ether, washed with 85 per cent ethanol, washed with distilled water until free of alkali, dried with sodium sulphate, than evaporated to dryness under reduced pressure. The residues were dissolved in cyclohexane, made to volume and read on the Beckman spectrophotometer. Wave length of 4530 A^{o} was used for measuring total carotenes and 3260 for vitamin A.

The extract from feces contained a material which absorbed light through the wave lengths 3000 to 5000 A^{o}. This absorption varied with the weight of the feces. Therefore the E values were corrected by subtracting the E values from the feces of the negative animals on the basis of weight of

feces.

RESULTS AND DISCUSSION:

Figure 7 presents the average absorption spectra of two or more carotene extracts. The male rat's feces collected during the four week experimental period for the negative group, and the groups fed vitamin A alcohol 4.6 micrograms daily, beta-carotene 7.0 micrograms, alpha-carotene 23.6 micrograms and raw carrot 20.0 micrograms of total carotenes per day were extracted and diluted to 25 ml. The corresponding female groups presented similar absorption curves. It is noted that the negative and the vitamin A alcohol groups both absorbed light throughout the 4000 to 5000 A^{o} area thus any spectrographic readings for carotene concentration must be corrected for this. In group 1, the average E_{4530} value on a 25 ml. extract, per g. of feces was 0.0131 for male negative control rats and 0.0151 for females; in group 2, 0.0164 for the male controls and 0.0170 for female controls. These values were used to correct all carotene determinations of feces reported.

This observation is in agreement with the work of Russell et al (37), Kemmerer and Fraps (35) and Wilson and co-workers (38). This absorption may be due to some material in the ration or some other excretory product, possibly resulting from the earlier sexual development of the treated rats. In either case a correction factor based on the weight of the feces would be more accurate than a total correction per rat. The vitamin A alcohol group presents further evidence for this as the feces extracts absorbed more light than those from the negative group. The food consumption of the former group was greater as was also the growth response and the weight of the feces. All groups fed carotene showed absorption maxima characteristic of carotenoids superimposed upon this broad band. Therefore, direct

spectrographic readings for total carotenes of feces should present true values if they are corrected by a blank prepared from the negative control group. Neither of the vitamin A alcohol groups showed any maxima at 3260 A° and in this region all groups including the negative controls absorbed a very high percentage of the light therefore no quantitative values for vitamin A excretion could be obtained in this research.

Table 6 presents the excretion and absorption data. The term absorption is used to mean the difference between the amounts of carotene fed and the amounts excreted. Some of the carotene not excreted may, however, be destroyed by oxidation rather than being digested and assimilated. The mechanical effect of feeding vegetables and comparing them to carotene in oil is practically removed in this research as all vegetables were ground to a fine pulp in a Waring blendor before oral feeding.

In general there was great variation im the amount of carotene excreted by individual rats fed at the same level and this was more marked at the low feeding levels than when the animals were receiving adequate amounts. The per cent carotene absorbed on intakes of 0.45, 0.70 and 1.03 micrograms daily, was higher for the female group than for the male but at all higher feeding levels there was no consistent variation between the utilization for male and female groups. On these feeding levels, six female and two male rats utilized all of the carotene. In all the groups in series 1 there were nine female and two male rats which did not excrete carotene. This may also indicate that at the lower levels of feeding the females utilized carotene more completely than the males.

There was no marked difference between the percentage of beta-carotene, alpha-carotene, raw or cooked spinach and carrot absorbed and in all cases the percentage was better than that reported by Fraps and Meinke (2, 42). The digestibility of the raw vegetables tended to be better than the cooked

and the carotene from spinach was absorbed as well as that from carrot.

Figure 8 presents the absorption spectra from wave lengths of 3000 to 3500 A^{o} of a vitamin A extract of a rat liver from the following groups; negative, beta-carotene 7.00 micrograms per day, alpha -carotene 23.6 micrograms, cooked carrot 20.0 micrograms and vitamin A alcohol 4.6 micrograms per day. All groups present approximately equal absorption which decreases as the wave length increases and shows no absorption maximum at 3260 A^{o} thus the presence of vitamin A was questionable. This method was compared to that of Carr-Price using one-half of the extract for each determination on liver from 10 of these animals and no blue color was formed indicating that no vitamin A was present.

The sensitivity of the absorption method was tested by weighing two portions of four grams each from a liver and adding vitamin A alcohol to one before carrying out the analytical procedure described. The percentage recovery ranged from 98 to 107 when 7 to 36 micrograms of vitamin A alcohol were added. Therefore this method should be satisfactory when a suitable blank is used.

The absorption, at wave length 3260 A^{o}, was in agreement with the work of Treichler, Kemmerer and Fraps (3) and which they termed pseudo vitamin A. The absorption spectra of these livers were studied from 2200 to 5000 A^{o} wave length and the absorption decreased as the wave length increased except for an absorption maximum between 2500 to 2600 A^{o}. With the carotene groups the extracts were pooled and reduced to a small volume for the reading of the absorption spectra from 4000 to 5000 A^{o} wave length and the absorption decreased as the wave length increased with no indications of maxima which might be considered characteristic of carotene. Therefore no carotene was stored in the liver when 20.0 micrograms per day was fed for a four week period.

SUMMARY AND CONCLUSIONS:

The digestibility of carotenes was studied on weanling rats fed beta-carotenes at levels of 0.45, 0.70, 1.03, 1.36, 2.00 and 7.00 micrograms per day, alpha-carotene at 2.36 and 23.60 micrograms, raw and cooked spinach and carrots at 3.82 micrograms of total carotenes and raw and cooked carrots at 20.0 micrograms total carotenes per day. The feces were collected for a 28 day period and analyzed. Liver storage of vitamin A was studied on groups fed the following; beta-carotene 7.0 micrograms, alpha-carotene 23.60 micrograms, vitamin A alcohol 4.6 micrograms and raw and cooked carrot 20.00 micrograms of total carotenes per day.

The absorption spectra of carotene extracts of the feces is presented from wave lengths of 3000 to 5000 A^{o}. Rat feces extracts show a broad continuous band which decreased in density with increasing wave length. When carotenes were fed, there was superimposed upon this broad band absorption maxima, characteristic of carotenoids. Total carotene determinations were made by spectrographic readings at 4530 A^{o} and corrections made for absorption not due to carotenes by using extracts from the feces of the negative controls on the basis of their weight.

There was no marked difference between the percentage of beta-carotene, alpha-carotene raw or cooked spinach and carrots absorbed. The range was from 61 to 91 per cent.

At low levels of feeding, there was a great variation in the utilization of carotenes within groups. Female rats absorbed carotenes better than males at the lowest levels of feeding.

The absorption spectra of extracts of rat livers were studied from wave lengths of 2200 to 5000 A^{o}. The extracts decreased gradually in density with increasing wave length except for a maximum between 2500 and 2600 A^{o}.

At the levels fed there was no storage in the liver of vitamin A or carotenes.

GENERAL SUMMARY:

Carotenes were studied under the following headings; 1. the alpha- and beta-carotene content of raw, cooked and frozen spinach and carrots, 2. the relative value of alpha- and beta-carotene, vitamin A alcohol, raw and cooked spinach and carrots for growth of the rat, 3. the fecal excretion and absorption of various carotenes and storage as vitamin A in the liver of the rat.

Spinach and carrots, purchased in the local market were analyzed spectrographically for total carotenes and alpha- and beta-carotene in the raw and cooked state and at various periods of frozen storage. Weanling rats on a vitamin A free ration were fed beta-carotene, alpha-carotene, vitamin A alcohol and raw and cooked spinach and carrots at various levels of intake for a 28 day period. The growth response, fecal excretion, absorption and liver storage of vitamin A were studied.

The total carotene content of raw spinach was 4.19 and 5.96 mg. per 100 g. of vegetable, carrots, 14.00 and 10.53 mg. for vegetables purchased in May and November respectively. The percentage beta-carotene was 76.4 and 81.6 for raw spinach and 66.2 and 76.4 for raw carrot respectively. Cooked spinach contained 6.33 and 6.92 mg. total carotene and cooked carrot 12.79 and 9.64 mg. per 100 g. of vegetable with a beta-carotene content of 86.5 and 94.1 for cooked spinach and 58.6 and 62.3 for cooked carrot. Frozen storage resulted in little change in total carotene content of either raw spinach or carrots in three months but losses up to 50 per cent occurred after 15 months storage. There was little change due to storage in total carotenes of the cooked product. Absorption spectra are presented for alpha- and beta-carotene in cyclohexane and for extracts of the raw and cooked spinach and carrots and these products after 10 months frozen storage.

Cooking produced some change in the absorption curves but frozen storage, of the raw products, for 10 months, caused a marked change in the absorption curves, indicating isomerization. The change in the cooked products were less significant, indicating that cooking stabilized the carotene content.

In the growth study of weanling rats, when all sources of vitamin A are calculated to micrograms of beta-carotene from the beta-carotene growth response curve, the ratio of beta-carotene to vitamin A alcohol, alpha-carotene, the total carotenes of raw spinach, cooked spinach, raw carrot and cooked carrot for male rats are 1.00: 0.45: 2.03: 2.08: 2.25: 3.18: 2.56 respectively and for female rats 1.00: 0.72: 5.90: 3.29: 3.64: 6.47: 3.29 micrograms of beta-carotene respectively.

The percentage carotene absorbed ranged from 61 to 91 but there was little difference between beta-carotene, alpha-carotene or the carotenes of raw or cooked spinach and carrots. At low feeding levels there was a great variation in utilization within groups and females in these groups absorbed carotene better than males. The absorption spectra of negative control rat's feces decreased in density with increasing wave length from 3000 to 5000 A^{o}. When carotenes were fed, there was superimposed upon this, absorption maxima, characteristic of carotenoids. The absorption not due to carotenoids necessitated the use of a suitable blank obtained, in this case, from the negative control group.

Rats stored no vitamin A in the liver at any levels fed. Absorption spectra of extracts of rat livers from wave lengths of 2200 to 5000 A^{o} decreased in density with increasing wave length except for a maximum between 2500 and 2600 A^{o}. This absorption would necessitate the use of suitable blanks for the spectrographic determination of vitamin A in rat livers.

TABLE 1. Specific Absorption Coefficients for Alpha- and Beta-Carotene in Cyclohexane Solution

Wave length	Specific Absorption Coefficient		
	Alpha-carotene	Beta-carotene	
A^{o}	l. per gm. cm.	l. per gm. cm.	
4340	159	159	coinsident points
4530	215	215	coinsident points
4700	175	175	coinsident points
4820	186	186	coinsident points
4780	203	183	far apart points
4900	108	158	far apart points

TABLE 2. Carotene Content of Raw and Cooked Vegetables Fresh and After Frozen Storage

Vegetable	Sample No.	Frozen Storage	Total carotene content				Carotene loss	Absorption Ratios		Beta-carotene
			4340A°	4530A°	4700A°	4820A°	4530A°	$\frac{4780A^{\circ}}{4820A^{\circ}}$	$\frac{4900A^{\circ}}{4820A^{\circ}}$	4820 - 4900A°
		months	mg. per 100 g. fresh weight				percent			percent
Raw spinach	1	0	4.39	4.19	3.97	3.91		0.992	0.812	76.4
		8	2.30	2.13	2.15	2.06	49.16	1.009	0.809	79.6
		15	1.89	1.70	1.73	1.60	59.43	1.021	0.789	71.7
	2	0	6.18	5.96	6.00	5.89		1.001	0.827	81.6
		3	5.90	5.56	5.51	5.37	6.71	1.005	0.831	88.6
		10	3.77	3.37	3.60	3.21	43.46	1.016	0.822	84.8
Cooked spinach	1	0	6.60	6.33	5.96	5.69		1.020	0.814	86.5
		8	6.13	5.92	5.92	5.83	6.48	0.999	0.852	97.1
		15	7.58	7.17	7.36	7.08	+13.27	1.001	0.840	92.2
	2	0	7.23	6.92	7.30	7.25		0.994	0.843	94.1
		3	6.87	6.70	6.71	6.59	3.18	0.997	0.848	95.0
		10	7.92	7.36	7.72	7.28	+ 6.36	1.004	0.845	94.2
Raw carrot	3	0	15.12	14.00	13.13	12.86		1.023	0.785	66.2
		8	10.62	10.31	10.38	10.40	26.36	1.018	0.773	65.3
		15	8.29	7.83	8.04	7.82	44.07	1.026	0.782	63.0
	4	0	11.00	10.53	10.93	11.04		1.010	0.793	76.4
		3	11.04	10.61	10.63	10.63	+ 0.76	1.013	0.783	69.1
		10	9.70	8.98	9.42	8.86	14.72	1.023	0.796	74.4
Cooked carrot	3	0	14.05	12.79	12.77	12.59		1.017	0.761	58.6
		8	13.50	12.86	12.93	12.76	+ 0.56	1.018	0.771	64.5
		15	12.77	11.52	11.92	11.28	9.93	1.027	0.772	64.9
	4	0	10.44	9.64	9.59	9.37		1.017	0.766	62.3
		3	11.60	10.75	10.84	10.63	+11.51	1.020	0.773	64.9
		10	11.39	10.16	10.66	9.98	+ 5.39	1.022	0.794	73.5

All figures represent triplicate analyses.
Samples 1 and 3 were purchased in May and 2 and 4 in November.
All analyses are expressed on the fresh-weight basis.
The total carotene fraction was analyzed spectrographically as a mixture of alpha- and beta-carotenes.

TABLE 3. Carotene Content of Vegetables.

Vegetable	Total Carotene	Beta-Carotene	Alpha-Carotene
	mg. per 100 g. of fresh weight	percent	percent
Raw spinach	5.96	81.6	18.4
Cooked spinach	6.92	94.1	5.9
Raw carrot	10.53	76.4	23.6
Cooked carrot	9.64	62.3	37.7

TABLE 4. Growth and Food Intake of Rats Fed Various Carotenes and Vitamin A Alcohol for 28 Day Period.

Supplement	Daily Intake	Males					Females					Males & Females Adjusted Gains
		Initial Weight	Food Intake	Gain in Weight	Adjusted Gain[2]	Beta-Carotene equiv.[3]	Initial Weight	Food Intake	Gain in Weight	Adjusted Gain[2]	Beta-Carotene equiv.[3]	
Scries 1.	γ	g.	g.	g.	g.	γ/day	g.	g.	g.	g.	γ/day	g.
0	0	48.6	254	59.0	75.0		42.8	240	57.4	77.7		76.3
beta-carotene	0.45	48.4	269	80.8	90.7		48.4	289	89.4	91.2		90.9
beta-carotene	0.70	48.0	288	98.4	100.9		47.0	293	92.8	92.6		97.7
beta-carotene	1.03	51.2	331	114.6	100.0		46.6	297	98.4	96.5		98.2
beta-carotene	1.36	48.2	323	121.2	109.1		47.2	310	104.0	96.9		103.0
beta-carotene	2.00	46.4	300	123.8	120.6		46.4	265	94.4	105.4		113.1
Vitamin A alcohol	0.46	51.2	299	104.4	102.8	1.03	49.4	281	87.0	92.2	0.64	97.5
alpha-carotene	2.36[4]	47.6	307	111.0	105.3	1.16	50.4	302	93.0	90.0	0.40	97.5
Raw spinach	3.82[4]	50.8	347	138.6	117.4	1.84	47.2	311	104.4	96.9	1.16	107.2
Cooked spinach	3.82[4]	50.2	331	129.8	114.9	1.70	46.8	295	97.0	95.9	1.05	105.3
Raw carrot	3.82[4]	47.8	324	118.6	106.0	1.20	49.8	298	93.4	91.8	0.59	98.8
Cooked carrot	3.82[4]	47.4	331	126.6	111.1	1.49	50.2	294	96.6	96.8	1.16	104.0
Series 2.												
0	0	42.8	209	57.6	90.5		41.4	222	69.2	96.5		93.3
beta-carotene	7.0	42.8	315	138.6	128.4		41.6	278	113.2	117.8		123.5
alpha-carotene	23.6	48.0	329	147.0	132.4		42.2	253	103.6	118.5		125.6
Vitamin A alcohol	4.6[4]	45.6	298	121.4	118.8	1.92	45.0	273	92.4	99.8	1.49	109.4
Raw carrot	20.0[4]	44.4	296	130.0	127.9		43.4	285	113.0	115.2		121.6
Cooked carrot	20.0[4]	43.8	304	132.6	122.1		42.8	284	111.6	114.0		120.7

Each figure represents the average of data on five animals.

2. Gains adjusted by covariance to correct for variation in initial weight and food consumption.
3. Beta-carotene equivalent was determined from the beta-carotene gain - dosage curve, Figure 1.
4. Total carotene as determined spectrographically.

TABLE 5. Covariance Table of Growth, Initial Weight and Food Intake of Rats for a 28 Day Period.

Source	D.F.	X S.S	XY S.S	XZ S.S	YZ S.S	Y S.S	Z S.S	Error of Estimate S.S	D.F	M.Sq	F.
Total	179	4,667	3,326	15,794	148,374	119,329	290,873				
Sex	1	84	1,177	1,313	18,387	16,492	20,502				
Treatment	17	1,114	12	6,675	67,015	63,746	120,167				
S x T	17	275	99	874	12,088	8,929	20,758				
Within Sex and Treatment (error)	144	3,194	2,039	6,932	50,884	30,162	129,446	9,998	142	70.4	
Treatment + Error	161	4,307	2,050	13,608	117,899	93,908	249,613	32,878			
Treatment								22,380	17	1,346	19.13 **
Sex + Error	145	3,278	3,216	8,245	69,272	46,654	149,948	14,494			
Sex								4,496	1	4,496	63.86 **
S x T + Error	161	3,469	2,137	7,806	62,972	39,090	150,204	12,239			
S x T								2,241	17	132	1.87

X = Initial weight in gms.
Y = Gain in weight in gms. in 28 days.
Z = Food intake in gms. in 28 days.
** The probabilities are 99/100 that sex and treatment effect growth response.

TABLE 6. The Fecal Excretion and Absorption of Carotenes by Rats.

Material Fed	Carotene Intake		Males			Females		
			Excretion		Digestibility	Excretion		Digestibility
	per day	Total in 28 days	Total in 28 days	Range		Total in 28 days	Range	
	γ	γ	γ	γ	percent	γ	γ	percent
beta-carotene	0.45	12.60	3.48	0-8.50	72.4	1.55	0-3.31	87.7
beta-carotene	0.70	19.60	6.70	0-13.18	65.8	2.70	0-8.20	86.2
beta-carotene	1.03	28.84	11.18	2.34-16.15	61.2	2.55	0-5.41	91.2
beta-carotene	1.36	38.08	5.19	2.43- 9.50	86.4	5.99	0-10.18	84.3
beta-carotene	2.00	56.00	22.12	7.59-35.59	60.5	17.32	12.09-25.53	69.1
beta-carotene	7.00	196.00	38.27	34.53-41.64	80.5	50.64	41.42-64.41	74.2
alpha-carotene	2.36	66.08	8.41	3.78-13.08	87.3	8.71	0-19.26	86.8
alpha-carotene	23.60	660.80	152.04	60.47-207.43	77.0	127.93	88.59-163.94	80.6
raw spinach	2.36	106.96	16.48	13.36-21.89	84.6	16.20	3.35- 26.08	84.8
cooked spinach	2.36	106.96	24.17	17.26-33.99	77.4	23.60	0-56.85	77.9
raw carrot	2.36	106.96	10.85	4.26-15.80	89.9	18.23	13.01-21.41	83.0
cooked carrot	2.36	106.96	18.19	1.14-29.78	83.0	17.90	6.38-26.41	83.3
raw carrot	20.00	560.00	105.88	86.43-141.24	81.1	124.73	88.73-152.65	77.7
cooked carrot	20.00	560.00	115.98	92.77-147.76	79.3	139.75	107.85-128.38	75.00

Each figure represents the average of data from five animals.
Vegetables were fed as total carotenes determined by chemical analyses.

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REFERENCES

1. Guggenheim, K. The biological value of carotene from various sources and the effects of vitamin E on the utilization of carotene and of vitamin A. Biochem. J. 38, 260-264, 1944.

2. Fraps, G. S. and W. W. Meinke. Digestibility by rats of alpha- beta- and neo-beta-carotene in vegetables. Arch. Biochem. 6, 323-327, 1945.

3. Treichler, R., A. R. Kemmerer and G. S. Fraps. The utilization of carotene and vitamin A in the rat. J. Nutrition, 24, 57-64, 1942.

4. Kemmerer, A. R., and G. S. Fraps. Constituents of crude carotene of carrots. Food Research 10, 457-460, 1945.

5. Kemmerer, A. R., G. S. Fraps, and W. W. Meinke. Constituents of the crude carotene of certain human foods. Food Research 10, 66-71, 1945.

6. Zscheile, F. P., B. W. Beadle, and H. R. Kraybill. Carotene content of fresh and frozen green vegetables. Food Research 8, 299-313, 1943.

7. Beadle, B. W. and F. P. Zscheile. Studies on the carotenoids. II. The isomerization of beta-carotene and its relation to carotene analysis. J. Biol. Chem. 144, 21-33, 1942.

8. Harper, R. H. and F. P. Zscheile. Carotenoid content of carrot varieties and strains. Food Research 10, 84-97, 1945.

9. Kemmerer, A. R., and G. S. Fraps. Constituents of carotene extracts of plants. Ind. Eng. Chem. Anal. Ed. 15, 714-716, 1943.

10. Mackinney, G., Leaf carotenes. J. Biol. Chem. 111, 75-84, 1935.

11. Devine, J., R. F. Hunter and N. E. Williams. The preparation of beta-carotene of a high degree of purity. Biochem. J. 39, 5-6, 1945.

12. Braude, R., A. S. Foot, K. M. Henry, S. K. Kon, S. Y. Thompson, and T. H. Mead. Vitamin A studies with rats and pigs. Biochem. J. 35, 693-707, 1941.

13. Kemmerer, A. R. Report on carotene. J. of A.O.A.C. 29, 18-24, 1946.

14. Strain, H. H., Carotene XI. Isolation and detection of alpha-carotene and the carotenes of carrot roots and of butter. J. Biol. Chem. 127, 191-201, 1939.

15. Zechmeister, L. Cis-trans isomerization and stereochemistry of carotenoids and diphenylpolyenes. Chem. Rev. 34, 267-344, 1944.

16. Zscheile, F. P. and J. W. Porter. Analytical methods for carotenes of lycopersicon species and strains. Anal. Chem. 19, 47-51, 1947.

17. Strain, H. H., and W. M. Manning. A unique polyene pigment of the marine diatom Navicula Torquatum. J. Am. Chem. Soc. 65, 2258, 1943.

18. White, J. W., F. P. Zscheile, and A. M. Brunson. The carotenoids of yellow corn grain. J. Am. Chem. Soc. 64, 2603-2606, 1942.

19. Lease, E. J., J. G. Lease, H. Steenbock, and C. A. Bauman. The biological value of carotene in various fats. J. Nutrition 17, 91, 1939.

20. Sherman, W. C. Chromatographic identification and biological evaluation of carotene from mature soybeans. Food Research 5, 13, 1940

21. Sherman, W. C. The effect of certain fats and unsaturated fatty acids upon the utilization of carotene. J. Nutrition 22, 153, 1941.

22. Smith, M. C. and L. Otis. Observations on carotene analyses of vegetables and fruits as a basis for prediction of their vitamin A value. Food Research 6, 143-150, 1941.

23. Kemmerer, A. R. and G. S. Fraps. Nature of carotenes in alfalfa. J. Am. Chem. Soc. 66, 305, 1944.

24. Deuel, H. J., Jr., E. Sumner, C. Johnston, A. Polgár, and L. Zechmeister. Stereochemical configuration and provitamin A activity III. All-trans-alpha-carotene and neo-alpha-carotene U. Arch. Biochem. 6, 157-161, 1945.

25. Kemmerer, A. R. and G. S. Fraps. Relative value of carotene in vegetables for growth of the white rat. Arch. Biochem. 8, 197-201. 1945.

26. Deuel, H. J., Jr., C. Johnston, E. Sumner, A. Polgár and L. Zechmeister. Stereochemical configuration and provitamin A activity I. All-trans-beta-carotene and neo-beta-carotene U. Arch. Biochem. 5, 107-114, 1944.

27. Deuel, H. J. Jr., E. R. Meserve, C. H. Johnston, A. Polgár, and L. Zechmeister. Re-investigation of the relative provitamin A potencies of cryptoxanthin and beta-carotene. Arch. Biochem. 7, 447-450, 1945.

28. Oser, B. L. and D. J. Melnick. Physiological availability to the rat of crude carotene in vegetables. J. Nutrition 30, 385-389, 1945.

29. Bacharach, A. L. Effect of ingested vitamin E (tocopherol) on vitamin A storage in the liver of the albino rat. Quart. J. Pharm. 13, 138-149, 1940.

30. Davies, A. W. and T. Moore, Inter-action of vitamins A and E. Nature 147, 794-800, 1941.

31. Harris, P. L., M. W. Kaley, and K. C. D. Hickman. Co-vitamin studies II. The sparing action of natural tocopherol concentrates on carotene. J. Biol. Chem. 152, 313-320, 1944.

32. Hickman, K. C. D., M. W. Kaley, and P. L. Harris. Co-vitamin Studies I. The sparing action of natural tocopherol concentrates on vitamin A. J. Biol. Chem. 152, 303-311, 1944.

33. Karrer, P., and H. Keller. Quantitative Bestimmung der tocopherole in verschiedenem ausgangsmaterialien, Helv. Chim. Acta 21, 1161-1169, 1938. C. A. 33, 1355-9, 1939.

34. Coward, K. H. Biological standardization of the vitamins, p. 22. W. Wood and Company, Baltimore, 1938.

35. Kemmerer, A. R., and G. S. Fraps. Digestibility of carotenes by rats and chickens. J. Nutrition 16, 309-315, 1938.

36. With, T. K. Absorption, metabolism and storage of vitamin A and carotene, with some remarks on the vitamin A requirements. Published by Einar Munksgaard and Oxford University Press, London. p. 1-269, 1940.

37. Russell, W. C., M. W. Taylor, H. A. Walker and L. J. Polskin. The absorption and retention of carotene and vitamin A by hens on normal and low fat rations. J. Nutrition, 24, 199-211, 1942.

38. Wilson, R. H., A. M. Ambrose, F. De Eds, H. J. Dutton and G. F. Bailey. The content and biological availability of carotene in raw and dehydrated carrots and other vegetables. Arch. Biochem. 10, 131-140, 1946.

39. Wagner, K. H. The excretion of vitamin A and beta-carotene in the feces and urine upon the administration of known amounts of vitamin A and beta-carotene. Z. klin. med. 137, 664, 1940. C. A. 37, 669[2], 1943.

40. Morgan, A. F., and L. S. Bentley. Storage of vitamin A in the livers of dogs fed beta-carotene or vitamin A. Fed. Proc. 3, 1, 1944.

41. Booher, L. E., and E. C. Callison. The minimum vitamin A requirement of normal adults. II. The utilization of carotene as affected by certain dietary factors and variation in light exposure. J. Nutrition 18, 459-471, 1939.

42. Fraps, G. S., and W. W. Meinke. Relative values of carotenes in foods as measured by storage of vitamin A in livers of rats. Food Research 10, 187-196, 1945.

43. Little, R. W., A. W. Thomas and H. C. Sherman. Spectrophotometric studies of the storage of vitamin A in the body. J. Biol. Chem. 148, 441-443, 1943.

44. Caldwell, A. B., G. MacLeod, and H. C. Sherman. Bodily storage of vitamin A in relation to diet and age, studied by means of the antimony trichloride reaction using a photoelectric colorimeter. J. Nutrition 30, 349-353, 1945.

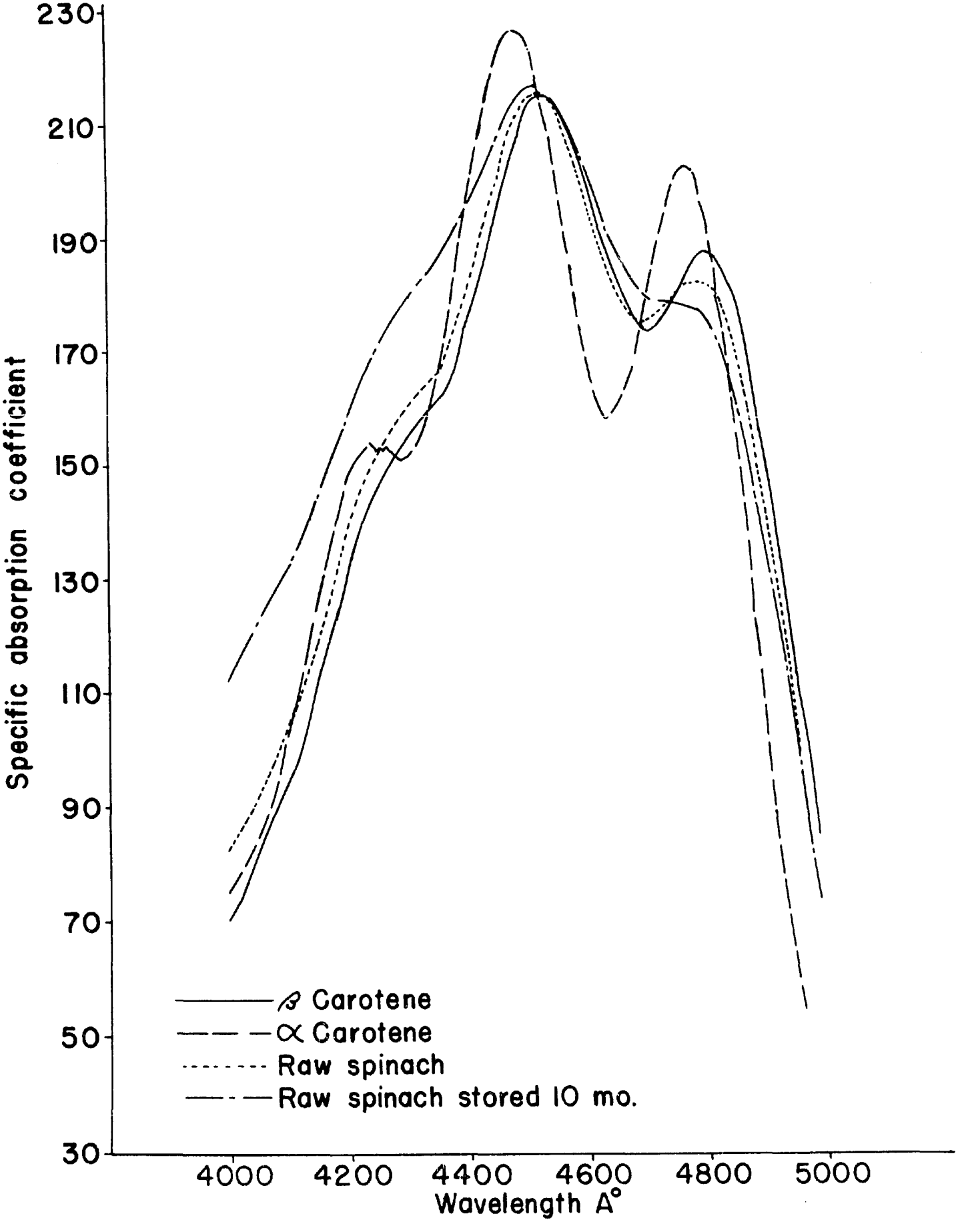
45. Rohrer, A. B. and H. C. Sherman. The bodily store of vitamin A as influenced by age and by food. J. Nutrition, 25, 605-609, 1943.

46. Campbell, H. L., M. Udiljak, H. Yarmolinsky and H. C. Sherman. Bodily storage of vitamin A in relation to diet and age studied by the assay method of single feedings. J. Nutrition 30, 343-348, 1945.

47. Lewis, J. M., O. Bodansky, F. G. Folk and G. McGuire. Vitamin A requirement in the rat. The relation of vitamin A intake to growth and to concentration of vitamin A in the blood plasma liver and retina. J. Nutrition 23, 351, 1942.

48. Callison, E. C. and V. H. Knowles. Liver reserves of vitamin A and their relation to the signs of vitamin A deficiency in the albino rat. Am. J. Physiol. 143, 444-452, 1945.

FIGURE 1. Effect of Frozen Storage on Absorption Spectra of Raw Spinach
(in cyclohexane)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∝ Carotene
Raw spinach
Raw spinach stored 10 mo.

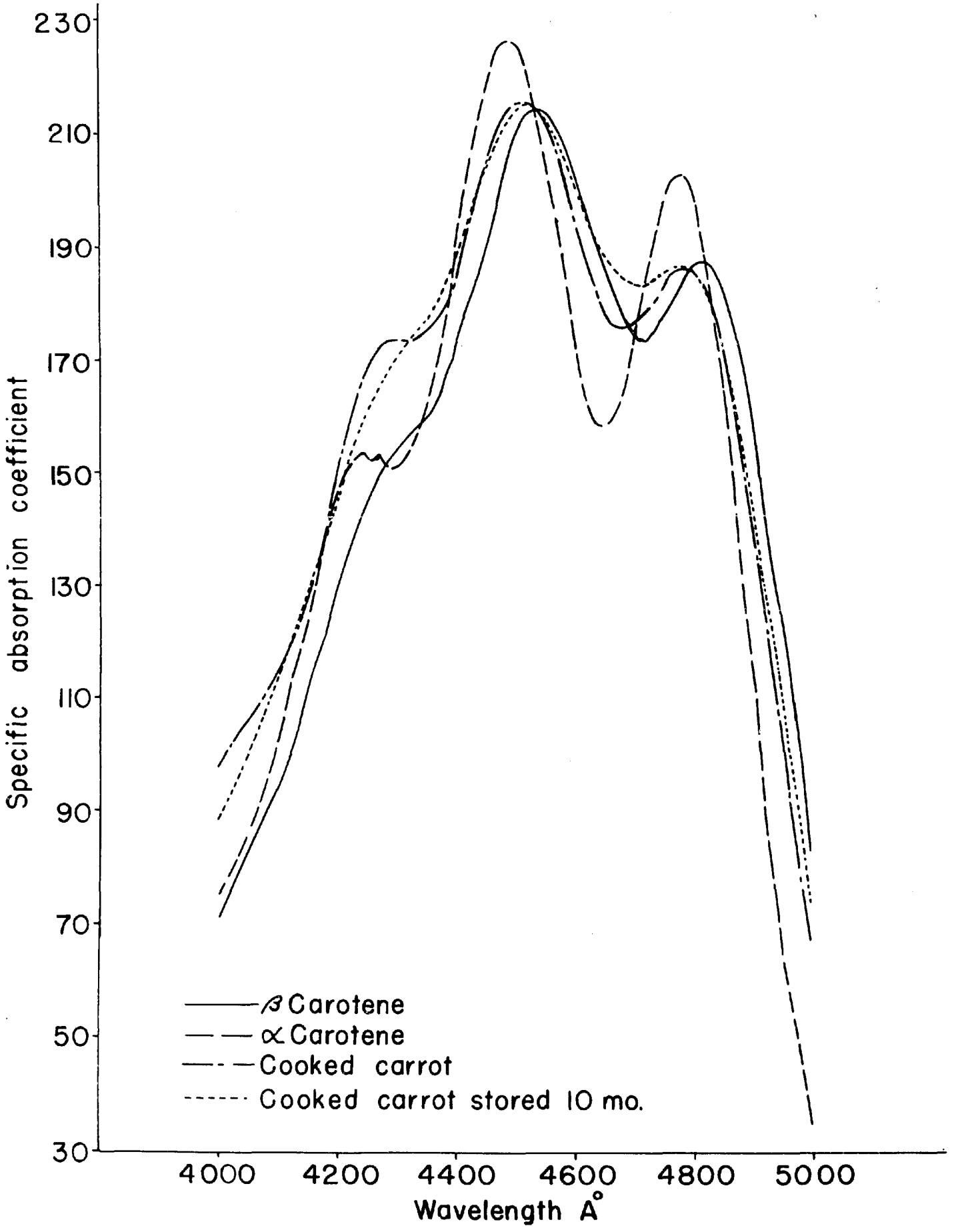

FIGURE 2. Effect of Frozen Storage on Absorption Spectra of Cooked Spinach (in cyclohexane)

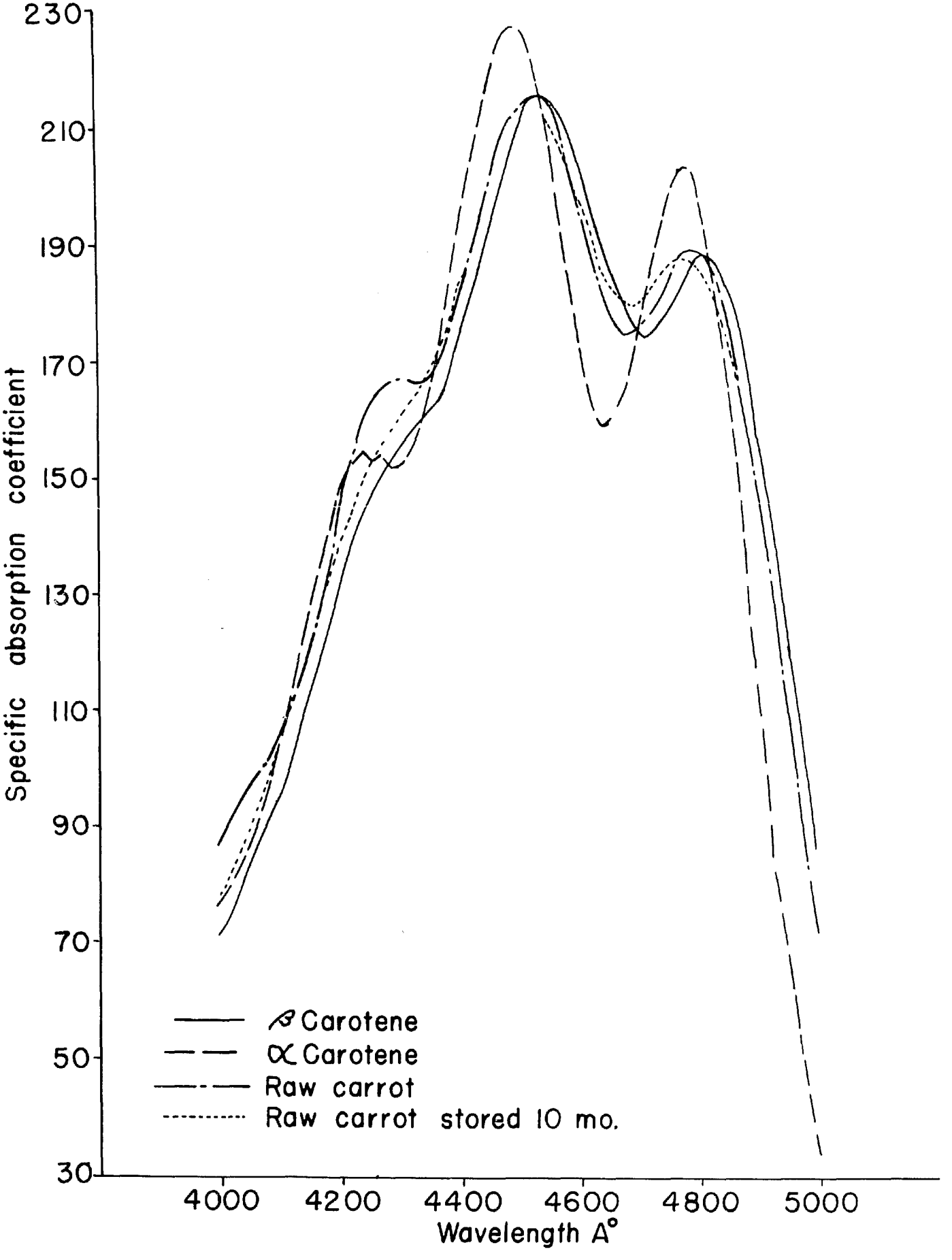

FIGURE 3. Effect of Frozen Storage on Absorption Spectra of Raw Carrot
(in cyclohexane)

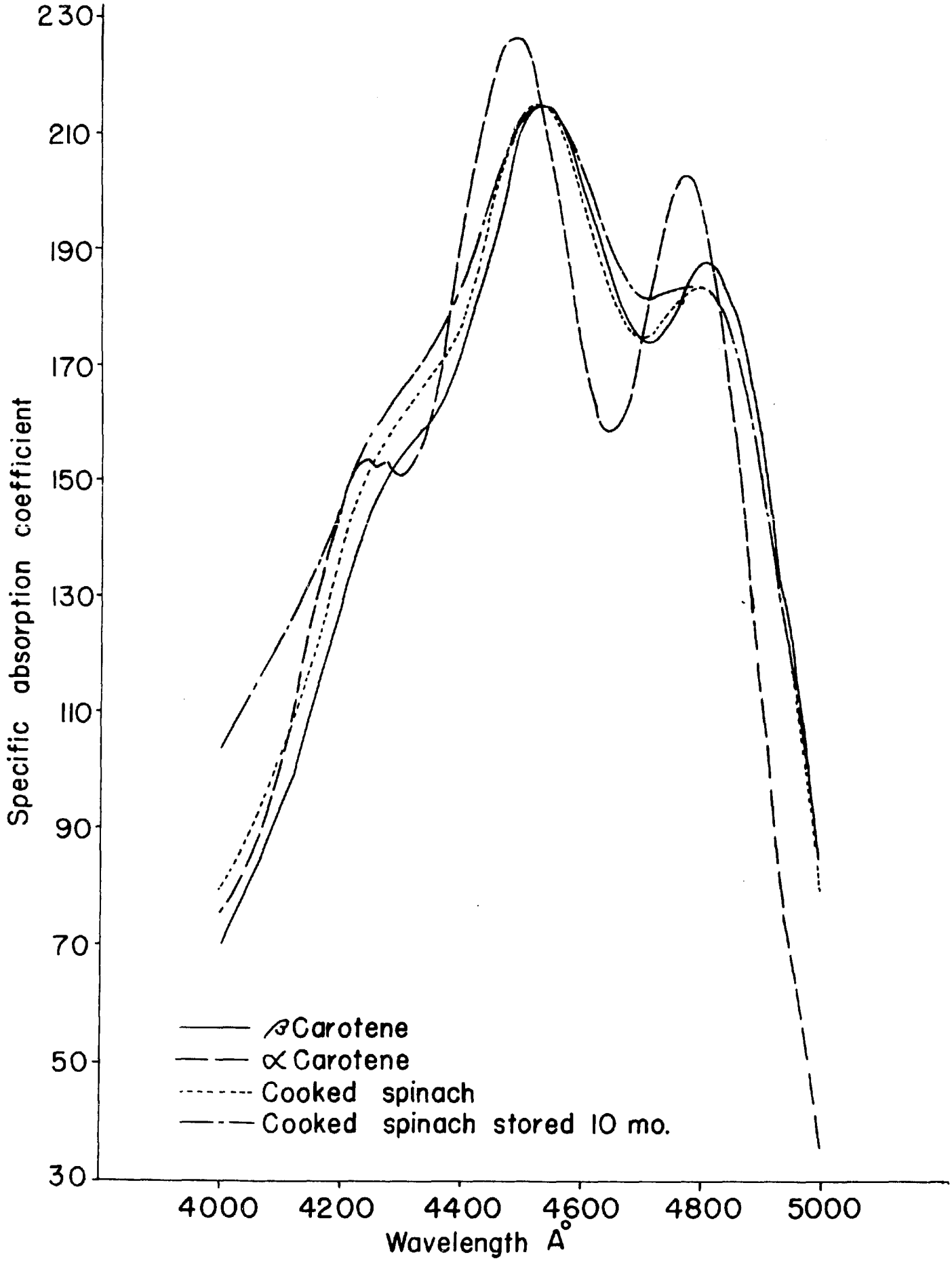

FIGURE 4. Effect of Frozen Storage on Absorption Spectra of Cooked Carrot
(in cyclohexane)

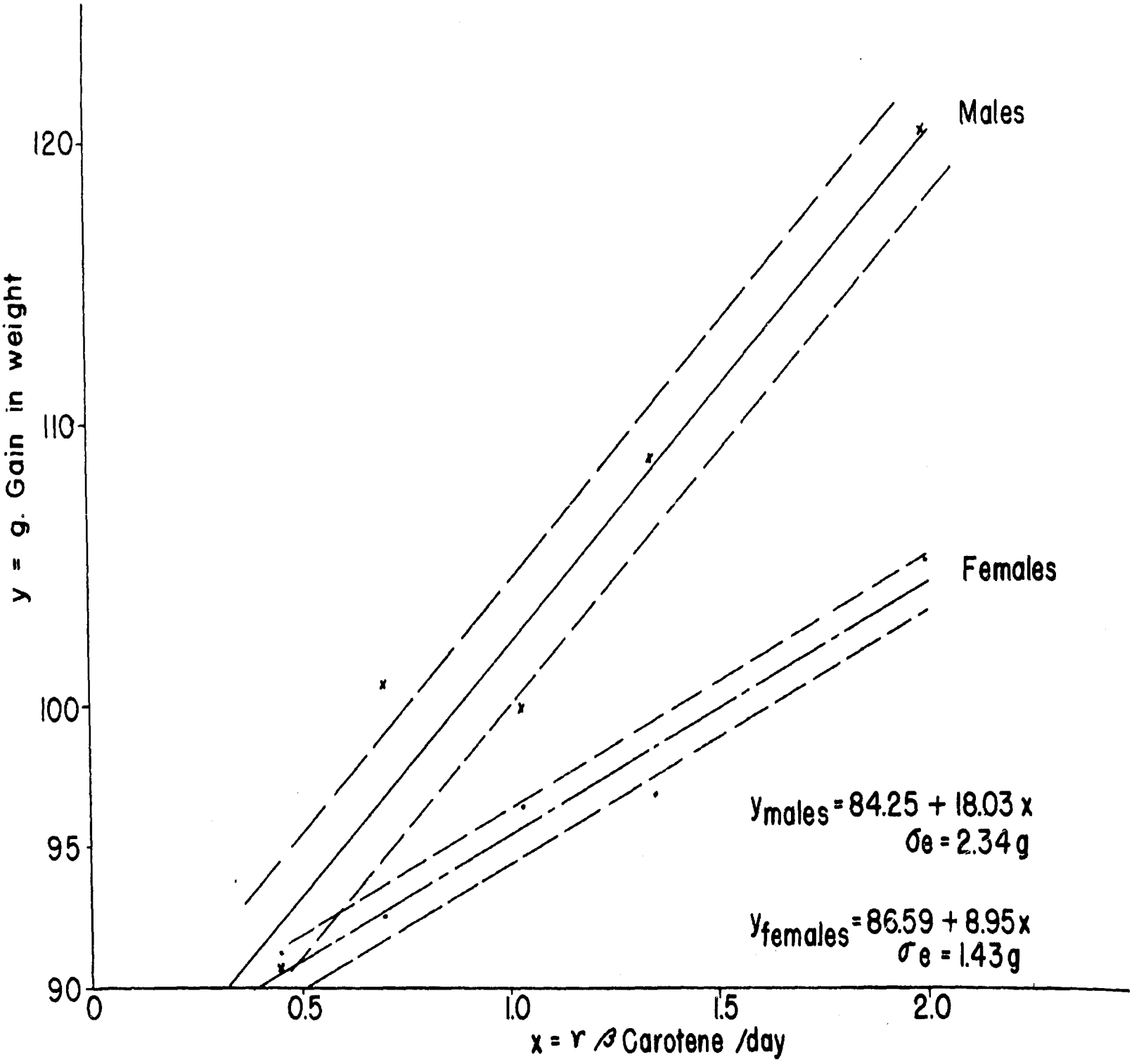

FIGURE 5. Relationship of Gain in Weight to Dosage of Beta-carotene for Male and Female Rats.

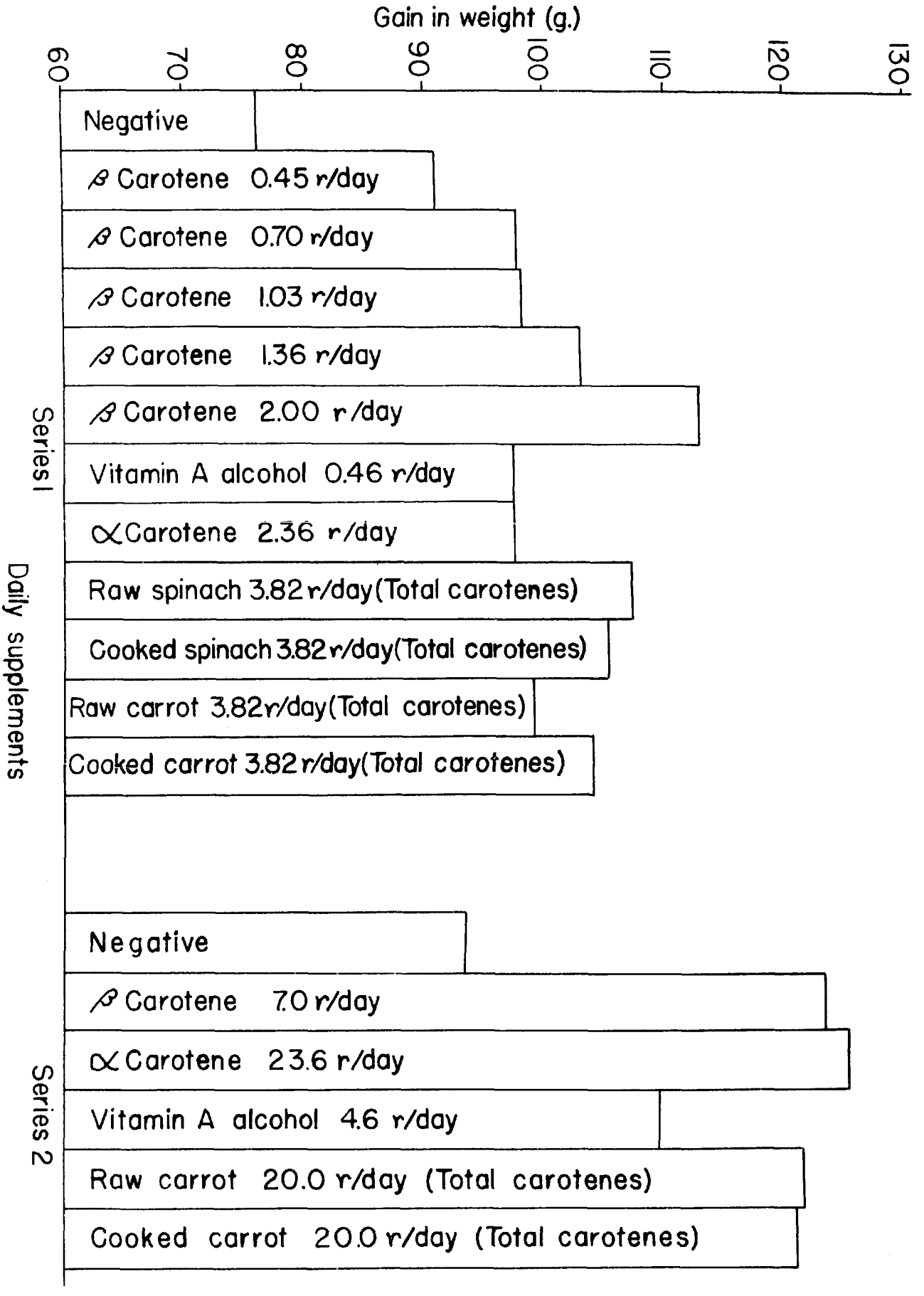

FIGURE 6. Relationship of Gain in Weight to the Various Supplements Fed to Each Group of Rats
(Average of 5 Males and 5 Females)

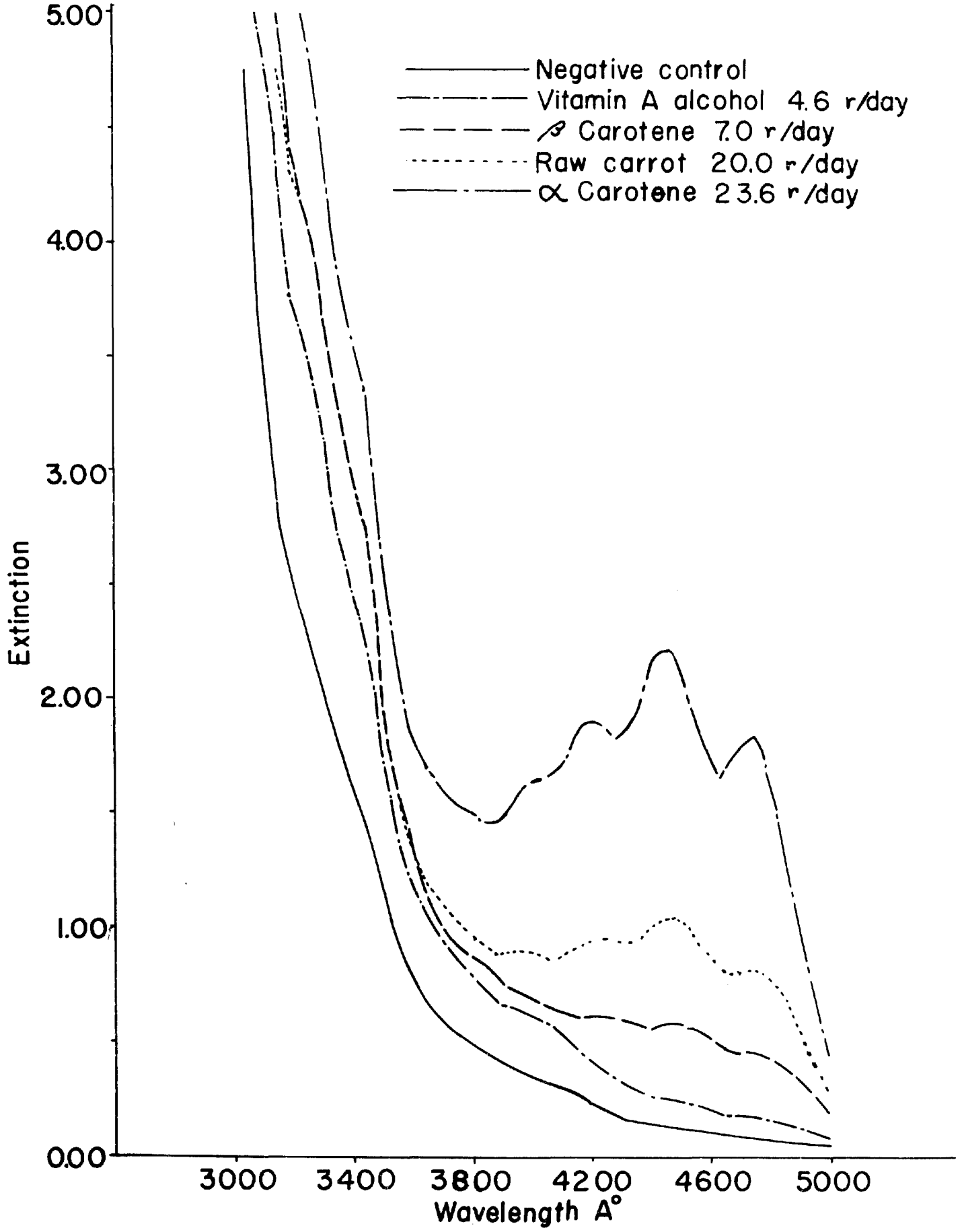

FIGURE 7. Absorption Spectra of Carotene Extract of Rat Feces

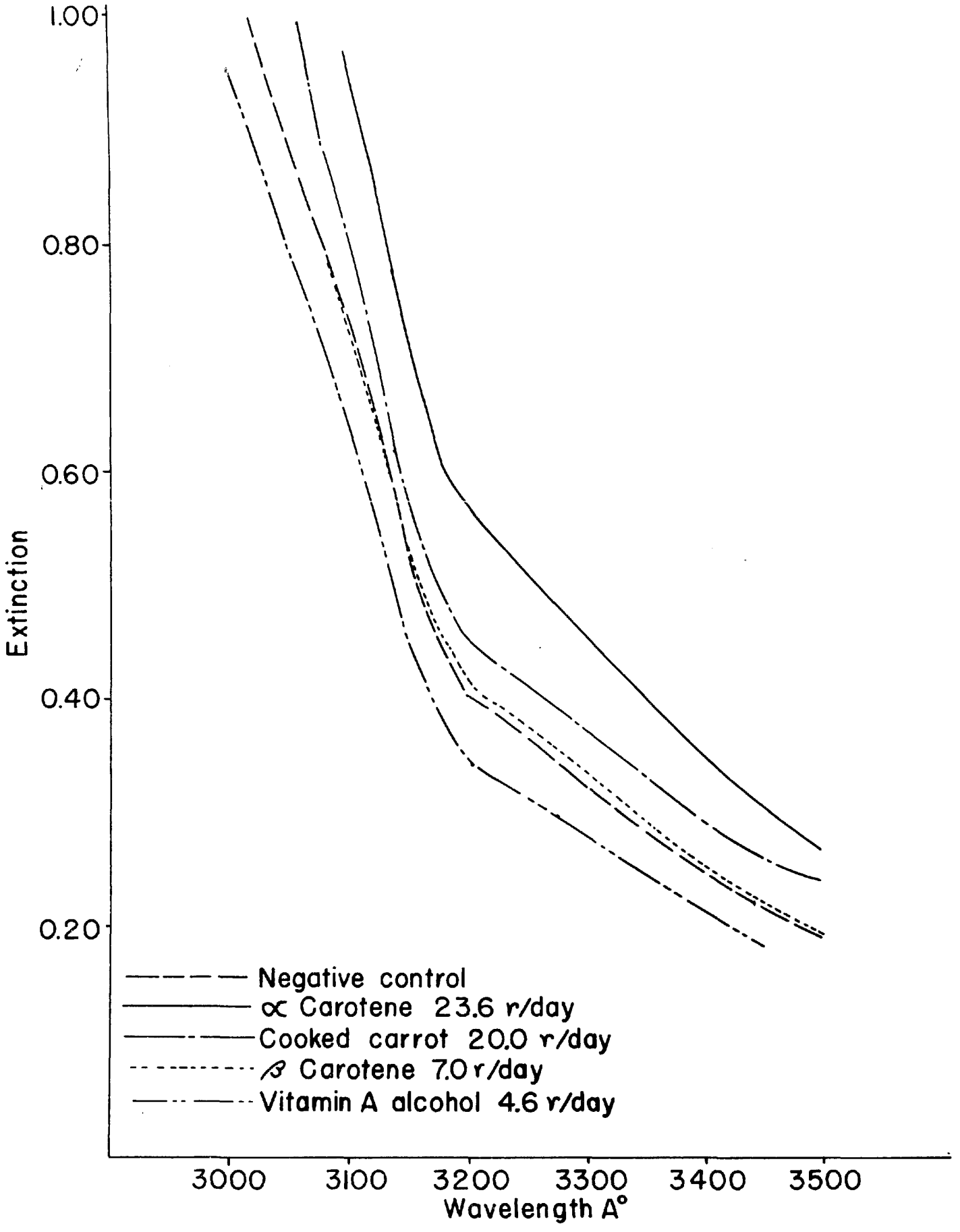

FIGURE 8. Absorption Spectra of Cyclohexane Extract of Rat Livers.