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Relation Between Vitamin A Potency and Carotene Content of Green Plant Tissue.*

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The finding that carotene is provitamin A for certain species raises the question as to whether a direct determination of the carotene content of plant tissue, coupled with information with regard to the vitamin A potency of isolated carotene, could be substituted for the extended biological assay. Euler, Demole, Karrer and Walker[1] report that a carotene determination in plant material discloses the magnitude of its vitamin A activity.

For the determination of carotene a colorimetric method was used, based on the original procedure of Willstatter and Stoll[2] but modified after critical study by one of us.[3] The vitamin A technique was essentially that employed by Sherman and has been referred to in an earlier publication.[4]

Alfalfa samples 45, 46 and 49 were taken from adjacent parts of the same field. Nos. 45 and 46 were dried in a mechanical drier by artificial heat within a few hours after cutting and No. 49 was sun-dried in the field. Samples 61 and 62 were artificially-dried and sun-dried, respectively, but from different parts of the country. A stock solution of carotene from carrots was prepared by dissolving a weighed amount of the crystalline substance (M.P. 169-171°, corr.) in ethyl laurate and olive oil. A dilution was made with olive oil every 2 weeks and in order to insure a constant level of carotene intake a colorimetric determination was made before each dilution. Hydroquinone was added to all solutions to the extent of 1 mg. per cc. as an antioxidant.

Table 1 displays the feeding levels which are in a comparable range as to rat growth response. The data, which are presented only in part, reveal that on the basis of carotene alone a smaller

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[1] Euler, H., Demole, V., Karrer, P., and Walker, O., *Helv. Chim. Acta,* 1930, **8**, 1078.

[2] Willstätter, R., and Stoll, A., *Untersuchungen über Chlorophyll,* Berlin, 1913.

[3] Chichester, D. F., Master's Thesis, Rutgers University, 1932.

[4] Russell, W. C., *J. Biol. Chem.,* 1929, **85**, 289.

TABLE I.

Sample	No.	Carotene content dry basis	Wt. daily supplement	Carotene in supplement as fed	Positive survivors†	Av. gain in body wt. 5-wk. test per.	No. animals
		%	mg.	γ(=0.001 mg.)	%	gm.	
Alfalfa	45	0.0037	21	0.71	89	14	9
"	46	0.0041	24	0.90	89	20	9
"	61	0.0059	15	0.83	90	18	10
"	62	0.0023	25	0.53	86	20	7
"	49	0.00017	150	0.23	73	15	11
Carotene	C_1			1.50	88	24	8
Alfalfa	61	0.0059	25	1.38	100	45	8

† Animals alive at 5 weeks and which had gained in weight.

amount of this substance in plant tissue is necessary to produce a given growth response in the rat than when isolated carotene is fed. This difference might be due to a greater potency of carotene in association with plant tissue, to the presence of some unknown substance which has growth-promoting properties in vitamin A deficient animals or to the conversion of part of the carotene in the plant tissue to vitamin A during the curing process. Thus the carotene contents of the supplements of the sun-dried samples Nos. 49 and 62, are less than those of the artificially dried, yet the animal response is of the same order. Furthermore, 1.5 international vitamin A units, (1.5γ), fed as carotene, caused an average growth response of 24 gm., yet practically the same number of units in a 25 mg. supplement of No. 61 resulted in an average response of 45 gm. There is the possibility that the method of analysis did not account for all of the carotene present, yet this is not likely in view of the critical study made of the procedure prior to the animal feeding trials.

Hence, while the carotene content of dried green plant tissue, determined colorimetrically, is a rough indication of vitamin A potency, the present data indicate that this determination cannot be substituted for the biological assay, and that the drying process may affect the relation of the carotene content to the animal response.