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Carotene and Vitamin A.

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Carotene was adopted as a temporary international standard for Vitamin A potency by the Permanent Standards Commission of the Health Organization of the League of Nations. Since then much interest has been shown in an exact determination of the value of this unit in terms of the Sherman Unit. The earlier literature assigns values between 2 λ and 20 λ of carotene as meeting the requirements for one Sherman Unit, while the more recent results of Polak and Stokvis[1] give values as low as 0.5 λ of carotene to one Sherman Unit. This paper is offered as a further contribution to this subject.

In preparing carotene from carrots by a modification of the method given by Schertz,[2] part of the carotene extracted was obtained in crystalline form and part remained in the concentrated petroleum ether extract. After removal of the petroleum ether by evaporation under proper conditions, a deep red carrot oil remained. Moore[3] found carrot oil potent as a source of Vitamin A. However, his work was not quantitative on the basis of the content of pigment. Hence, the questions arose, whether or not the pigment which remained in the carrot oil was as potent a source of Vitamin A as that which crystallized, and whether there was any growth-promoting factor in carrot oil other than that due to its carotene content when all the color in the carrot oil is considered as carotene. To determine this biologically, 2 test solutions were prepared as follows:

Solution 1. Crystalline Carotene Dissolved in Wesson Oil: A sample of carotene isolated from carrots was found to have a purity of 79% when tested by a modification of the potassium dichromate comparison method of Palmer,[4] based on the earlier results of Willstätter and Stoll. 5.1 mg. of this carotene were dissolved in 100 cc. of Wesson Oil, thus giving the oil an actual carotene content

[1] Polak, A., and Stokvis, J. A., *Arch. neerlandais de physiol. de l'homme et des animaux*, 1931, **16**, 542.

[2] Schertz, F. M., *J. Agr. Research*, 1925, **30**, 469.

[3] Moore, T., *Biochem. J.*, 1929, **23**, 803.

[4] Palmer, L. S., Carotinoids and Related Pigments, Chemical Catalog Co., Inc., New York, 1922, 259.

of 0.001 mg. (1 λ) in each drop from a pipette standardized to deliver 40 drops per cc.

Solution 2. Carrot Oil Diluted with Wesson Oil: Carrot oil prepared by the method outlined above was tested by diluting samples with petroleum ether and comparing with 0.2% $K_2Cr_2O_7$ solution. It was found to have color equivalent to 2.72% carotene.* The carrot oil was diluted 9:1 with Wesson Oil and when this was diluted with petroleum ether it gave a color equivalent to 0.27% carotene. 1.5 g. of this diluted carrot oil was further diluted to 100 cc. with Wesson Oil, thus giving a carotene content, as determined colorimetrically, equal to that of Solution 1, that is, 1 λ per drop (0.025 cc.) of oil. The original color of these 2 dilute solutions of carotene was retained throughout the complete biological test period.

Biological Tests. Albino rats† about 4 weeks old and weighing about 45 gm. were fed on a diet deficient in Vitamin A until they had definitely ceased gaining weight. This period varied from 6 to 9 weeks. The diet was composed as follows: Casein (inactivated) 20 gm., irradiated olive oil‡ (for Vitamin D) 15 gm., cornstarch (free of Vitamin A) 50 gm., salt mixture (McCollum, No. 185) 5 gm., yeast (Harris Medicinal) 10 gm. This was served as a very thick paste made by adding 50 cc. of water.

After the rats had definitely ceased gaining weight for about 2 weeks daily supplements of varying quantities of solutions 1 and 2, described above, were administered orally, by pipette, standardized to deliver 0.025 cc. per drop. Solutions 1 and 2 were further diluted with Wesson Oil in order to permit the administration of smaller quantities of carotene. Tables I and II give the average gains per week for the first 4 weeks and for the 8 weeks following the beginning of the administration of the solutions. The average gain for the first 4 weeks is also given in the tables because this is the method used by some of the British investigators. The average gain during

* In making colorimetric determinations of this type it is important to have the oil so diluted that at least 98% of the solvent is petroleum ether. Otherwise a correction should be made, as the color intensity of carotene in oil is several times that of an equal quantity dissolved in petroleum ether and the test is based on the color intensity of carotene in petroleum ether. This point has not been generally recognized, and it is planned to make it the subject of a later communication.

† Wistar strain, bred at the Institute of Pathology.

‡ 300 cc. olive oil in an open flat dish 35 cm. square irradiated for ½ hour at a distance of 40 cm. from the burner of a Cooper-Hewitt mercury vapor quartz lamp.

TABLE I.
Solution 1. Each rat given crystalline carotene dissolved in Wesson oil.§

Amt. carotene (mg.)	Aver. gain per week during first 4 wks. (gm.)	Aver. gain per week during 8 weeks (gm.)
.00025	Lost	Died in 4 weeks
.00025	,,	,, ,, 3 ,,
.00025	,,	,, ,, 3 ,,
.0005	3.5	2.8
.0005	5.0	2.9
.00075	6.2	3.6
.001	5.8	4.6
.001	9.0	5.8
.002	16.3	9.6
.002	8.5	6.6
.002	8.6	7.0
.003	10.0	7.0
.003	10.5	8.1
.004	16.7	8.3
.004	9.2	7.5
.008	17.7	14.0

§Control rats that received daily 8 drops of Wesson oil alone, lost weight and died in from 3 to 6 weeks after the end of the depletion period.

TABLE II.
Solution 2. Carrot oil diluted with Wesson oil.

Amt. carotene (mg.)	Aver. gain per week during first 4 wks. (gm.)	Aver. gain per week during 8 weeks (gm.)
.00025	Lost weight	Lived 4 weeks
.0005	6.2	3.9
.0005	5.1	3.8
.00075	6.6	4.0
.001	11.2	6.2
.001	8.0	5.6
.001	6.5	5.0
.002	10.2	6.0
.002	8.0	5.8
.003	13.2	6.5
.004	11.7	6.5
.004	13.5	9.7
.008	16.0	10.6

the 4 weeks was always greater than during the 8 weeks. The results show that of each solution a quantity containing 0.0005 mg. (0.5 λ) of carotene was the minimum that just satisfied the requirements for a Sherman unit of Vitamin A, that is, an average gain of 3 gm. per week for 8 weeks. The number of animals on the lower doses was small because the previous work of other investigators did not lead us to expect the potency found in the solutions here described.

The results show that the color in freshly prepared carrot oil, when considered as carotene, is equal in Vitamin A potency to an equivalent amount of crystalline carotene. This not only confirms the opinion that carotene is the only pigment of consequence in

carrot oil, but also indicates first, that carotene is probably the only growth-promoting factor of the order of Vitamin A in carrots, and second, that the potency of carotene is not increased by the presence of any of the other constituents of carrot oil.

The results also confirm those reported by Polak and Stokvis,[3] who found carotene to be much more potent as a source of Vitamin A than had previously been reported by other investigators. Should it become generally recognized that 0.5 λ of carotene satisfies the requirements for a Sherman Unit of Vitamin A, then one International Unit of Vitamin A (1 λ of pure carotene) may be considered as equivalent to 2 Sherman Units.

The extreme variation (0.5 to 20.0 λ) in the amount of carotene required for one Sherman Unit of Vitamin A, as reported by various investigators, is not in every case readily explainable. In some instances the lower potencies may have been due to factors such as the use of impure carotene preparations, loss of carotene by oxidation during the preparation of the solution or during the biological tests, or to methods of administration which resulted in faulty assimilation. In the tests here reported precautions were taken against these 3 factors. The solutions were made up on a basis of the actual carotene content as determined colorimetrically; periodic tests showed that the original color of the 2 dilute carotene solutions was retained throughout the biological test period; and, to our knowledge, the most desirable method of administration was used. In some preliminary tests we found that solutions of carotene in ethyl laurate and in olive oil aerated at 120° C. for 6 hours became completely decolorized in a few weeks. Similar results have been reported by others. It is possible that there was more or less loss of carotene in this way in some of the cases reported where higher doses of carotene were required to promote growth. Further research may also show considerable variation in the percentage of carotene assimilated depending upon the type of solvent and method of administration.