CXVI. VITAMIN A AND CAROTENE.

X. THE RELATIVE MINIMUM DOSES OF VITAMIN A AND CAROTENE.

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RECENT research has demonstrated that the richest vitamin A concentrates obtainable react in the antimony trichloride test some 10–20 times more intensely than carotene. Moreover, since the colorimetric, spectroscopic and analytical data given by concentrates prepared from a variety of liver oils tend to approach constant values, there are strong grounds for assuming that the concentrates represent more or less pure vitamin A [Karrer, Morf and Schöpp, 1931, 1, 2; Moore, 1931, 1; Heilbron *et al.*, 1932].

With such knowledge at our command it becomes possible to enquire into the relative efficiency of utilisation of the two substances in the animal, as indicated by the relative minimum curative doses. Since large dietary excess of carotene may lead to the excretion of the pigment in the faeces [Moore, 1931, 2; Ahmad, 1931] the writer at one time suggested that the conversion of carotene might be inefficient even at levels of intake approaching the minimum dose, and predicted that vitamin A concentrates much more active than carotene should be obtainable. This possibility seemed to be supported by the statement of Karrer, Morf and Schöpp [1931, 1] that a concentrate from *Hippoglossus* liver, which possessed biological activity 10 times greater than that of carotene, had been prepared, and also by the claim of Heilbron *et al.* to have obtained growth responses with vitamin A concentrates at levels much below the reputed minimum dose of carotene.

A review of the literature, however, reveals that the minimum doses quoted by different workers both for vitamin A and carotene vary over a wide range. In the case of carotene the dose necessary to restore growth was originally given by Euler, Euler and Hellström [1928] as 5 γ (0.005 mg.), but recently Polak and Stokvis [1931; 1932] and also Goldblatt and Barnett [1932] have obtained responses at levels down to 0.5 γ . In the case of vitamin A Euler and Karrer [1931] first gave 0.5 γ as the minimum dose of a vitamin A concentrate (*Hippoglossus*), a result in fair agreement with preliminary work by the writer [Moore, 1932] in which rather less pure specimens of rat-, pig- and turbot-liver oil concentrates were found to have minimum doses of the order of 1–2 γ . Karrer, Morf and Schöpp [1931, 1]¹, however, referring to work done by Euler, gave 0.5 γ as the dosage necessary for normal (not minimum) growth, while in a later communication Euler [1932] has quoted the same work of Karrer, Morf and Schöpp as having given the dose of vitamin A concentrate necessary for normal

¹ These authors originally gave the dose for normal growth as 5 γ but this was subsequently corrected to 0.5 γ .

growth as 0.1 γ , and has advanced fresh evidence that another concentrate, particulars of which are not given, could restore good growth at 0.3 γ . Even lower doses have been found to be effective by Heilbron *et al.* [1932]. In a limited number of experiments these workers have observed good growth on 0.1 γ , with definite signs of improved condition on 0.025 γ .

While small intrinsic differences in the biological activities of specimens of carotene and vitamin A concentrates prepared in different laboratories must almost certainly exist, it seems impossible that the extent of such differences could account for the wide range of minimum doses reported by different workers. On the other hand it is well recognised that results obtained in the biological test may be influenced by several variable factors, including differences in (1) the basal diet, (2) the experimental rats, both in regard to the initial vitamin A reserve and general state of health, (3) the medium used for diluting the test dose, and (4) the methods adopted in measuring growth responses, and in defining "normal" and "minimum" growth. Under these circumstances it is obvious that any attempt to deduce a ratio comparing the activities of the two substances from published data collected at random must be almost meaningless. To reach a satisfactory conclusion it is plainly necessary to carry out experiments in which the two substances are given in parallel experiments to closely matched groups of rats. This procedure has already been adopted in preliminary work, as a result of which it was inferred that vitamin A must possess not more than twice the biological activity of the international standard specimen of carotene. In certain of these experiments, however, a complication arose through the partial deterioration of the test solutions of vitamin A during the period of dosing, while on the other hand the standard carotene cannot be regarded as of the highest attainable purity. For these reasons the results of the preliminary experiments will not be described in detail in the present communication, but attention will be confined to a final experiment which is not open to criticism on these grounds.

EXPERIMENTAL.

24 young rats, 20 albino and 4 from piebald stock, all bred under conditions ensuring reasonably low initial vitamin A reserves, were fed upon a diet consisting of Glaxo caseinogen AB 15 %, rice starch 80 %, salt mixture 5 %, dried yeast 10 % (additional), radiostol 1 drop per rat per week. At the end of the preliminary depletion period (49–59 days) the rats were carefully paired for dosing either with carotene or a vitamin A concentrate. Each rat (with the exception of 1 pair, Nos. 10 and 10 *a*) was matched with a litter-mate of the same sex, the degree of similarity of the preliminary weight curves being taken into account when alternative choices were available.

Carotene. The carotene used was a highly purified specimen of β -carotene (2000 y.u., 350 b.u. per mg.) made available through the kindness of Prof. Richard Kuhn, to whom my best thanks are due.

Vitamin A concentrate. The concentrate was prepared from turbot-liver oil by alcoholic saponification, followed by the removal of sterols by crystallisation and precipitation by digitonin. It gave 3500 b.u. per mg. (= Carr-Price value 65000) and had $E_{1\text{ cm.}}^{1\%}$, 328 $m\mu$ = 950. As a basis for calculating the probable amount of "pure" vitamin A present in this concentrate the intensity of the absorption band at 328 $m\mu$ has been chosen as being less liable to personal error in observation than the blue value. In describing the richest concentrate Carr and Jewell [1933] have given the value $E_{1\text{ cm.}}^{1\%}$, 328 $m\mu$ = 1600. Taking this value

as a provisional maximum for "pure" vitamin A it is obvious that 1 γ of pure vitamin A was present in 1.68 γ of the concentrate.

Dosing. Coconut oil (B.D.H.) was chosen as a diluent for the test doses on account both of the great stability of solutions of carotene and vitamin A in this oil and of the absence of interference by the oil with the antimony trichloride

Fig. 1. Comparison of the biological activities of β -carotene (left-hand side of fig.) and vitamin A (right-hand side). The vitamin A was given as turbot-liver oil concentrate, 1.68 γ being assumed to contain 1 γ of vitamin A. Paired rats are indicated by having the same number, the rat receiving vitamin A being in each case distinguished by the addition of the letter *a*. The rats having (P) after their numbers were from piebald stock, all the others were albinos.

test [Norris and Church, 1930]. Two stock solutions of carotene containing 1 γ and 0.1 γ per drop were made up by dissolving a weighed amount of the pigment in chloroform, adding a small amount of oil, evaporating off the chloroform on a water-bath under diminished pressure and finally diluting to the required concentration by adding more melted oil. These stock solutions were kept in wide-necked bottles and stored throughout the experiment in a refrigerator.

Twice weekly amounts sufficient for immediate requirements were chipped off and melted in small conical flasks. Two similar stock solutions of the vitamin A concentrate, containing 1.68γ (1γ "pure" vitamin A) and 0.168γ were made up in the same way.

The stability of the test solutions was checked by observations of yellow value in the case of the carotene solutions, and of blue values in the case of the vitamin A solutions, the tests being applied to the portions of solution which had been in use for the preceding few days immediately before replacing by fresh solution from the stock bottles. No instances of deterioration were observed in the present experiments.

Dosing was carried out at the levels of 3γ , 1γ , 0.3γ and 0.1γ in the case of carotene, and at the corresponding levels of concentrate, 3 rats being used at each dosage. Treatment was continued for about 10 weeks, or until the previous death of the animal. The weight curves obtained are shown in Fig. 1.

DISCUSSION.

From the weight curves it will be seen that the responses caused by the corresponding doses of β -carotene and the vitamin A concentrate were very similar at all levels. Thus with 3γ of carotene or $3 \times 1.68\gamma$ of the concentrate good growth resulted in every rat. At the 1γ level growth was less rapid, while one rat (4) receiving carotene declined and died suddenly after having grown steadily for 7 weeks. At the 0.3γ level the responses were irregular, but again the behaviour of paired rats was very similar. Thus rats 7 and 7 *a* showed a temporary restoration in growth followed by sudden decline and death, rats 8 and 8 *a* decline and death, rats 9 and 9 *a* slow growth. At the 0.1γ level all the rats died with the exception of 12 *a*, which survived in a weak condition for more than 10 weeks without showing much change in body weight, although xerophthalmia of gradual increasing severity persisted throughout the whole period.

It is plain, therefore, that β -carotene possesses biological activity equal to that of about the same weight of "pure" vitamin A, although the latter has a blue value some 10–20 times greater¹. In other words the blue unit of carotene is equivalent to some 10–20 b.u. of vitamin A. If, moreover, we accept the data given by Carr and Jewell for their richest concentrate as applicable to pure vitamin A then it must follow that carotene is utilised in the body no less efficiently than preformed vitamin A, or at least that the difference in favour of vitamin A is only fractional, since the present experiments were not designed to detect any small difference in activity, such as might result from a loss of part of the carotene molecule during conversion.

The low minimum dose found for β -carotene raises another question which may deserve brief mention. Kuhn and his colleagues have reported that various other substances, *e.g.* α -carotene [Kuhn and Brockmann, 1931], γ -carotene [Kuhn and Brockmann, 1933], β -hydroxycarotene [Kuhn and Brockmann, 1932] and a new pigment isolated by Winterstein [1933] from the fruit of *Gonocaryum pyroforme* also possess biological activity. While no positive reasons for doubting these claims can be advanced the evidence put forward would be considerably strengthened if the biological activities were to be compared with that of β -carotene at levels approaching the minimum dose. This precaution would

¹ The exact ratio depends to some extent on the conditions under which the comparison is carried out. Thus the vitamin A blue tends to be diminished in the presence of oils, the carotene blue to be increased.

perhaps be most desirable in the case of Winterstein's pigment. This substance was separated by the Tswett absorption technique from a source which also contained α - and β -carotenes, in a yield so small (1.7 mg.) that extensive processes of purification could not be carried out. Since the dose given in biological tests was as much as 18 γ it is obvious that some 5 % of β -carotene present as an impurity could account for the observed activity.

SUMMARY.

The biological activities of β -carotene and of a vitamin A concentrate were compared in parallel experiments. From calculations based on the amount of pure vitamin A presumed to be present in the concentrate it would appear that, contrary to views widely held, carotene is utilised in the body as efficiently as preformed vitamin A at levels approaching the minimum dose.

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