THE HUMAN EXCRETION OF CAROTENOIDS AND VITAMIN A[1]

WE have examined the intestinal excretion of vitamin A, carotene and xanthophyll in human subjects, in order to provide an objective measure of the dietary content of these substances, and to determine upper limits for their intestinal absorption.

Weighed samples of fresh feces were ground with equal weights of anhydrous sodium sulfate, and the powdery mixture was soxhletted with n-pentane. The extract was saponified under nitrogen in 6 per cent. KOH in methanol. The non-saponifiable fraction was partitioned between benzine and 90–95 per cent. methanol, to separate epiphasic "carotenes" from hypophasic "xanthophylls." Both fractions recombined, or portions of the original extract, were brought into chloroform for the antimony chloride reaction with vitamin A. All concentrations were measured with the Pulfrich Photometer by methods previously described.[2] The average recovery of added carotene or xanthophyll was 80 per cent., of added vitamin A 74 per cent. The qualitative efficiency of the extraction and fractionation procedures was checked spectrophotometrically.

A number of earlier investigations of carotenoid excretion have underestimated the time required for these substances to pass through the intestine.[3] The excretion of single large doses of carotene or xanthophyll in cottonseed oil ordinarily begins within 24 to 48 hours, rises to a maximum in the third to fifth days, and ceases in the fifth to seventh days or even later, depending upon the size of the dose. A single determination of feces carotenoid yields information, therefore, not on the vagaries of a single meal, but on the level of carotenoid ingestion over a considerable period. This factor stabilizes the measurements, and adds greatly to their general usefulness.

EXCRETORY LEVELS

Single samples of feces from 20 subjects eating well-balanced unrestricted diets contained 13.5–328 (av. 122) μgm. of carotenes, and 4.72–47.2 (av. 26.0) μgm. of xanthophylls per gram fresh weight. One subject whose total carotenoid excretion on an unrestricted diet was followed for 30 days yielded very similar results: 11.7–286 (av. 142) μgm. of carotenes and 8.9–105 (av. 29.0) μgm. of xanthophylls per gram of feces. The daily excretion of this subject averaged 74.5 grams; in 3 other subjects it averaged 53, 103 and 165 grams. The average water content varied between 71 and 75 per cent. The carotenoid concentration is relatively independent of the day-to-day bulk of the feces, even when this is increased by adding agar to the diet. It is curious that the carotene content of these "normal" feces is about equal to that of green leaves. The relative proportions of carotenes and xanthophylls, however, are the reverse of those in the leaf, apparently due to the preferential absorption of xanthophylls in the human intestine.

On passing from a high-carotenoid diet to one designed to contain the equivalent of about 100 I.U. of vitamin A daily, the feces carotenoid concentrations in 11 subjects dropped to 0.69–5.47 (av. 2.99) μgm. of carotenes and 0.79–3.73 (av. 2.00) μgm. of xanthophylls per gram. In two subjects whose diets were controlled more rigidly for long periods, the level of excretion fell still lower, to 1.22–1.70 μgm. of carotenes and 0.94–1.18 μgm. of xanthophylls per gram. The feces carotenoids provide, therefore, a sensitive index of the carotenoid levels of the diet.

Even at the lowest intake levels the feces carotenoids are virtually entirely of dietary origin. On passing from a high to a low-carotenoid diet, the rate of excretion falls to the new level within 5 to 7 days. When carmine is fed with the last high-carotenoid meal, the concentration of excreted carotenoid continues to fall no more than a day longer than carmine continues to appear in the feces. Furthermore, at the lowest excretory levels the daily excretion is still only about 70–80 per cent. of the daily intake, measured by direct extraction of 24-hour replicas of the diet. Only negligible amounts of feces carotenoids, therefore, can originate in intestinal organisms or by excretion from internal reserves through the intestinal wall.

PROPORTIONS EXCRETED

The excretion of carotene in cottonseed oil (the leaf mixture containing about 90 per cent. β- and 10 per cent. α-carotene) was determined in two subjects. Five experiments were performed, with ingestion periods ranging from a single dose to 10 days of regular feeding, and daily intake varying between 1.88 and 19.6 mgm. The fractions excreted in these experiments were 61.3, 60.8, 57.3, 69.7, and 49.3; average 59.7 per cent.

In similar experiments in which crystalline leaf xanthophyll in cottonseed oil was fed at the rate of 10.1 mgm. daily, the average excretory rate in one subject was 10.5 μgm. per gram feces, and the total

[1] This research was supported in part by a grant to G. W. from the Josiah Macy, Jr. Foundation. Halibut liver oil and general dietary supplements were generously supplied by the Abbott Laboratories of North Chicago, and carotene by the S. M. A. Corporation of Chicago.

[2] G. Young and G. Wald, *Amer. Jour. Physiol.*, 131: 210, 1940.

[3] *Cf.* H. E. C. Wilson, S. M. Das Gupta and B. Ahmad, *Ind. Jour. Med. Res.*, 24: 807, 1937.

excretion during an ingestion period of 16 days was 8.3 per cent. of the intake.

When vitamin A in halibut liver oil was fed at a level of 23.7 mgm. per day (about 76,000 I.U.), the excretory rate in 6 subjects averaged 20.6 μgm. (about 66 I.U.) per gram feces. The total excretion in two subjects during ingestion periods of 8 and 16 days was 3.97 and 2.74 per cent. of the intake. At still higher intake levels the excreted fraction rose sharply. Conversely, when the daily intake was reduced to about 25,000 I.U., the excretory rate in one subject fell to about 1/10 of its previous value, and a second subject excreted no measurable amount of vitamin A at all. In no case could we obtain a test for vitamin A from the feces of subjects on unrestricted, unsupplemented diets. It appears from these experiments that, unlike carotene or xanthophyll, vitamin A is not excreted until the intake reaches a threshold value, well above all ordinary dietary levels; and that above this the fraction excreted rises with the intake.

De has performed experiments similar to these in the rat.[4] He found the excretion of vitamin A in halibut liver oil to be 3 to 5 per cent. over a wide range of intake levels. The average excretion of carotene in oil was 42.5 per cent., or only about two thirds of that in our experiments on human subjects.

In bioassay experiments on rats, vitamin A is almost exactly twice as potent as an equal weight of β-carotene.[5] If one assumes that the fractions of vitamin A and carotene not excreted are absorbed—the excretion in any case sets an upper limit to the absorption—such apparent absorptions explain adequately the relative potencies of these substances in the rat.[4] By a parallel argument, due to its low apparent absorption, carotene in human bioassay should be at best only about 40 per cent. as effective as an equal weight of vitamin A; and should possess only about two thirds the potency now assigned to it on the basis of rat assays. Some confirmation of this conclusion has already appeared in human bioassay experiments.[6]

It is not now known whether β-carotene is converted to vitamin A *in vivo* by symmetrical cleavage, according to the equation

$$C_{40}H_{56} + 2\,H_2O \longrightarrow 2\,C_{20}H_{30}O \qquad (1);$$

or by stepwise degradation to yield a single molecule of vitamin A. In the former instance carotene and vitamin A should be about equally potent *in vivo;* in the latter, carotene should be only about half as potent as the vitamin. The fact that in rats the different potencies of these substances are explained by their differential absorptions implies that *following absorption* they are about equally effective, and is strong presumptive evidence for the operation of equation (1). The present experiments indicate a similar possibility in man.

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[4] N. K. De, *Ind. Jour. Med. Res.*, 24: 751, 1937.
[5] T. H. Mead, S. W. Underhill and K. H. Coward, *Biochem. Jour.*, 33: 589, 1939.
[6] L. E. Booher, E. C. Callison and E. M. Hewston, *Jour. Nutrition*, 17: 317, 1939.

CORRELATION OF ACTIVITY PER UNIT WEIGHT OF TOBACCO-MOSAIC VIRUS WITH AGE OF LESION

IT has been assumed by investigators working with plant viruses that each virus particle attains its full biological activity as soon as it is formed and that any increase in activity is due to an increase in the number of these infectious units. This would infer that all virus samples prepared under identical conditions should have the same activity per unit weight of virus regardless of the source of the sample. Examination of the literature reveals little experimental evidence in support of this assumption. What evidence there is has been derived from activity measurements of crude plant juice. Such measurements have given no information regarding the possible presence of infectious particles of different sizes and weights.

The development of the ultracentrifuge for virus isolation and purification, together with improvements in the local lesion method for measuring the activity of tobacco-mosaic virus, has made it possible to study virus samples containing known weights of virus protein. This technique has been applied to a study of the virus content and relative activity of preparations extracted at various intervals after inoculation. It was soon found[1] that under nitrogen-deficient conditions there was a falling off in activity per unit weight of virus. This finding led to a critical examination of the biological activity of virus in newly formed lesions.

Turkish tobacco plants (*Nicotiana tabacum* L.) were grown in nutrient sand cultures and supplied a complete nutrient solution. When about 6 inches tall, the plants were inoculated by rubbing over the entire upper surface of one mature leaf on each plant with a suspension of tobacco-mosaic virus (*Marmor tabaci* H.). At 5-day intervals, inoculated leaves from representative plants were harvested, frozen and then minced. The cold juice was cleared of insoluble materials by low-speed centrifugation and then ultracentrifuged. The sediment was suspended in phosphate buffer, cleared by low-speed centrifugation and again ultracentrifuged for 1 hour. Virus-protein content was calculated from the nitrogen content of the suspension of the sediment from the second ultra-

[1] E. L. Spencer, *Plant Physiol.*, 16: 229, 1941.