Carotene : a Rachitogenic Factor in Green-feeds

In a previous communication[1], evidence was presented indicating the presence of a rachitogenic factor in green-feeds. Experiments have shown that, for the rat on a rachitogenic ration, the bone ash response to a fixed dose of vitamin D is depressed by increasing doses of a green-feed extract in the manner indicated in Table 1.

Table 1

Group (18 rats per group)	Daily dose per rat: Vitamin D* (I.U.)	Daily dose per rat: Dried grass (mgm.)†	Average ash in dry fat-free femur (per cent)
1	0·075	0	33·2
2	0·15	0	37·9
3	0·15	1·75 (1·2 μgm. carotene)	38·1
4	0·15	3·5 (2·4 ,, ,,)	36·3
5	0·15	7·0 (4·8 ,, ,,)	35·6
6	0·15	14·0 (9·6 ,, ,,)	32·9
7	0·15	28·0 (19·2 ,, ,,)	31·6

* Amount dosed in 0·1 gm. olive oil solution.
† The equivalent weight of dried grass dosed as the chloroform extract in 0·1 gm. olive oil solution.

Statistical analyses carried out on these and on the results of eight other similar trials indicated a linear relationship between response (per cent bone ash) and log dose. A method was devised whereby an approximate estimate of the potency of the rachitogenic factor in a green-feed extract or concentrate could be obtained, an arbitrary unit being provisionally defined as "the amount which, when dosed daily to the rat on the McCollum 3143 rachitogenic ration plus a daily supplement of 0·15 I.U. vitamin D, would depress the bone ash response to that normally produced by a daily supplement of 0·10 I.U. vitamin D". Thus it became possible to follow quantitatively the fate of the active principle throughout the processes involved in its isolation. By chromatographic fractionation of a petroleum ether extract of dried green oats, the rachitogenic factor was isolated and identified as carotene.

The amount of carotene in each dose of extract described in Table 1 is indicated by the figures in brackets. The results obtained by dosing increasing amounts of carotene (B.D.H., 90 per cent β — 10 per cent α) to rats receiving the McCollum 3143 ration and a daily supplement of vitamin D are presented in Table 2.

Table 2

Group (24 rats per group)	Daily dose per rat: Vitamin D (I.U.)	Daily dose per rat: Carotene (μgm.)	Average ash in dry fat-free femur (per cent)
1	0·05	0	32·4
2	0·10	0	34·3
3	0·20	0	38·7
4	0·15	0·781	34·9
5	0·15	3·125	34·3
6	0·15	12·5	32·6
7	0·15	50·0	31·2
8	0·15	100·0	30·6

That vitamin A probably has a similar rachitogenic effect is seen from the results of a preliminary trial shown in Table 3.

Table 3

Group (12 rats per group)	Daily dose per rat: Vitamin D (I.U.)	Daily dose per rat: Vitamin A (I.U.)	Average ash in dry fat-free femur (per cent)
1	0·15	0	34·1
2	0·15	50	31·8

Analyses of green-feeds in this laboratory have indicated a carotene content as high as 840 μgm. per gm. of dry matter, so that animals grazing on such fodder have a high carotene intake—the equivalent of 1–2 million I.U. of vitamin A per day in the case of sheep, and for cattle about ten times this amount. In addition to this, an appreciable quantity of carotene appears to be synthesized by micro-organisms of the ileum and cæcum[2].

The identification of carotene as a rachitogenic factor for rats and the relatively large amounts of this substance present in green-feeds suggest that the rachitogenic effect of winter green-feed on sheep is due to the high carotene intake at a time of the year when the vitamin D status of the animal is normally low. This hypothesis is to be tested by sheep trials during the coming winter. The further possibility of a high carotene intake having other adverse effects on the health of farm animals is also being investigated.

A detailed account of this work, together with comprehensive statistical analyses carried out by Dr. A. H. Carter, of the Animal Research Station, Ruakura, will be presented elsewhere.

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[1] Grant, A. B., *Nature*, **168**, 789 (1951).
[2] McGillivray, W. A., *Brit. J. Nutrit.*, **5**, 223 (1951).

Tryptophane Metabolism in Rice Moth Larva (*Corcyra cephalonica* St.)

Earlier workers[1], who observed the excretion of the tryptophane metabolites, kynurenine and xanthurenic acid, by the rat and the rabbit under conditions suggestive of vitamin B deficiency, failed, however, to realize its physiological significance. It was not until Lepkovsky *et al.*[2] isolated and identified the pigment metabolite, xanthurenic acid, from the urine of pyridoxine-deficient rats receiving extra-dietary tryptophane, that the role of this vitamin in the metabolism of the amino-acid was unequivocally established. Since then, a number of reports have appeared of the conversion of tryptophane to xanthurenic acid, kynurenine, hydroxykynurenine, etc., by pyridoxine-deficient rats, rabbits and dogs[1,3]. In 1945, Sarma[4] found that the rice moth larva (*Corcyra cephalonica* St.), maintained on a pyridoxine-deficient diet containing added tryptophane, excreted yellow-coloured fæces. This was about the first and only report on the role of pyridoxine in tryptophane metabolism in insects and established a striking parallel in the metabolism of the amino-acid between mammals and insects. Pyridoxine had earlier been shown by the same author[5] to be an essential vitamin for the growth of the larva. The excretory pigment, however, was not characterized; but it was shown that it was not identical with xanthurenic acid, from the negative test it gave with iron salts. Tryptophane has also been shown to be the precursor of the pigments, ommochromes, found in the eyes and tissues of certain insects, and the production of the pigments appears to be controlled by genes[6]. Kynurenine and hydroxykynurenine, which are normal intermediates in this conversion, and kynurenic acid, which is derived from kynurenine by oxidative deamination