Letters to the Editor

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Occurrence of Antirachitic Vitamin in Green Plants

As is well known, antirachitic substances arise through irradiation with ultra-violet light. It might be presumed, therefore, that green plants, which are constantly exposed to the light of the sun, would become rich sources of the antirachitic vitamin. However, green plants, or parts thereof, have hitherto been found to be poor in the above-mentioned vitamin. This may, perhaps, be ascribed to the fact that the plant material has been prepared in an unsuitable manner prior to examination. It may also be, however, that the irradiation with sunlight has not been so intense as would have been supposed. As the summer of 1933 in southern Norway was unusually rich in sunny days (sunny days recorded in Oslo: May 25, June 28, July 30 and August 30) we considered it of interest to examine whether green plants this summer would show a larger vitamin D content than is usually the case.

For this investigation was used meadow-hay, consisting of Gramineæ and some clover, which was rapidly dried by a special quick-drying process (at 68° C. for 2 hours—a process which it is now intended to use on a larger scale). The hay was afterwards pulverised. The hay powder had a fresh, green colour, and yielded by extraction with ether in a Soxhlet apparatus 4 per cent of a deep green, ointment-like extract. Daily doses of four milligrams of this extract brought about a satisfactory cure of rickets (method: Poulsson and Løvenskiold[1]). The ether extract had, in other words, the same antirachitic effect as a high quality cod liver oil, containing about 250 Oslo units of vitamin D per gram. This corresponds to 0·25 unit vitamin D per gram of hay powder.

Some time ago, Kon and Booth[2] stated that vitamin D in butter showed a marked difference from the vitamin D found in cod liver oil and that obtained by ultra-violet irradiation of ergosterol; whereas 80 per cent of the first was lost by the usual saponification, this is not the case with vitamin D from the other two sources. We considered it of interest to examine whether this also applied to vitamin D in the above-mentioned ether extract of green plants. We brought about saponification by means of alcoholic potash-lye. 8 gm. ether extract yielded 0·508 gm. ether-soluble unsaponifiable matter, that is, 6·25 per cent. This was diluted with inactive arachis oil until a quantity was obtained equal to that of the ether extract from which we started, namely, 8 gm. Of the solution thus obtained, it was necessary to use 20 milligrams in order to obtain the same antirachitic effect as was found in the ether extract before saponification. Vitamin D in green plants shows, accordingly, the same characteristics as Kon and Booth[2,3] have described for vitamin D in butter.

We found it of interest, at the same time, to record the tintometric reading of the above-mentioned solution of the unsaponifiable matter, in arachis oil. This was found to be 10 blue units (which corresponds to a high quality cod liver oil). However, the tintometric reading, thus recorded, is probably not due to vitamin A, but to carotene, the precursor of vitamin A, as the chlorophyll was removed by the saponification, and the unsaponifiable matter showed a very pronounced yellow-red colour. 1,200 yellow and 20 red units were recorded as self-colour on Lovibond's tintometer.

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[1] Poulsson and Løvenskiold, *Biochem. J.*, **22**, No. 1; 1928.
[2] Kon, S. K., and Booth, R. G., *Biochem. J.*, **27**, 1189, 1302; 1933.
[3] Zucker and Barnett, *Proc. Soc. Exp. Biol. Med.*, **20**, 375; 1922–23.

Assay of Vitamin A

IN carrying out a series of assays, by the curative method, of the vitamin A content of various samples of fish oils and dried milk, it was found that in a large proportion of cases the weight curve did not give a reliable indication of the state of depletion of the vitamin A stores of the animal, and that increase in weight after administration of a supplement could not always be ascribed to its vitamin A content.

The experimental data obtained in these assays appeared to conflict with current ideas regarding the special influence of vitamin A on growth. An investigation was therefore undertaken to ascertain (1) whether growth does in fact cease in vitamin A deficiency, and (2) the real significance of the loss in weight which is generally described as 'cessation of growth'.

The evidence which has been obtained shows that when vitamin A is the only known factor absent from the diet, there is no cessation of growth, interpreting growth as increase in size. This has been determined by measurements of length of the body in the live animal, and by comparison of the lengths of the bones, measured post-mortem, with Donaldson's values for the standard rat. It would appear that vitamin A has no greater claim to be considered essential for growth *per se* than any other of the many factors which are responsible for increase in weight.

The characteristic loss in weight, which has been termed 'cessation of growth', appears to be due entirely to pathological conditions arising from the vitamin A deficiency. Even in animals killed at a stage when they are still increasing in weight, these conditions may be found on macroscopic examination.

The diversity of the pathological symptoms which may arise during the preliminary 'depletion' period makes it impossible to secure uniformity in the experimental animals at the beginning of the test period. This constitutes a source of error which makes the curative method of vitamin A assay of doubtful value. It seems probable that the various discrepancies so frequently reported in such assays may find their explanation in the above observations.

The results of this investigation, which were presented at a meeting of research workers at Aberdeen on December 18 last, will be published in detail at an early date.

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