XIII. THE FEEDING OF XANTHOPHYLL TO RATS ON A DIET DEFICIENT IN VITAMIN A.

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ALTHOUGH the theory of the identity of vitamin A with one of the yellow plant pigments has been abandoned [Drummond and Coward, 1920], most of the experimental work leading to this conclusion has been based upon carotene, while xanthophyll appears to have received less direct attention. This is somewhat surprising in view of the fact that xanthophyll is present in plant tissues in excess of carotene, but is probably accounted for by the well-known fact that vitamin A accompanies carotene rather than xanthophyll in the phase test. Drummond [1919] fed crystalline carotene to rats in the proportion of 0.003 % of the total diet and found it to be ineffective as a source of vitamin A.

Stephenson [1920] removed the carotene of butter fat by adsorption with charcoal without affecting its content of vitamin A.

The evidence is not so complete in the case of xanthophyll. Rosenheim and Drummond [1920] studied the effect of a xanthophyll preparation on rats, but as this material was impure and an indication of growth was obtained, the results were rightly considered inconclusive. On the other hand, Palmer and Kempster [1919] were able to remove xanthophyll from the diet of growing chicks without adversely affecting their health, powers of reproduction, or the virility of their offspring through two generations. The tissues of these birds and their eggs were devoid of carotene and xanthophyll.

Signs of returning interest in the carotenoid pigments are indicated in the paper of Drummond, Channon and Coward [1925]. These authors found that vitamin concentrates prepared from the unsaponifiable fraction of cod-liver oil are always more or less yellow coloured, a fact which led them to consider the possibility of carotenoid pigments being involved. Lycopin and carotene were thus tested by these investigators and found to be inactive as a source of vitamin A, whilst a feeding experiment with xanthophyll was not undertaken as their material was not pure. It therefore seemed of interest to prepare a pure specimen of crystalline xanthophyll and to study its effect when fed in known amount to rats on a diet deficient in vitamin A compared with that of a potent source of this vitamin such as cod-liver oil.

EXPERIMENTAL.

The crystalline xanthophyll was prepared from young nettle leaves by the method of Jørgensen and Stiles [1917] with certain modifications. Air-dried powdered nettle leaves were sieved so as to remove stalks and midribs and placed in the hot room at 40° for 3 days. The powder was then quickly re-ground, transferred to a large desiccator and dried over sulphuric acid *in vacuo* for a further 4 days. In the preliminary extraction with acetone 2 litres of solvent were used for each 500 g. of nettle powder as the filtrate still appeared considerably coloured after the 1½ litres recommended by Jørgensen and Stiles had been added. It was also found advantageous to recover the solvent held by the material after filtration under reduced pressure by expression in the hand press. By so doing more than a litre of extract was recovered from 1500 g. of the original material. Otherwise the method of Jørgensen and Stiles was closely followed. The crude xanthophyll was recrystallised three times from methyl alcohol, a little water being added to assist crystallisation and the flask placed in the refrigerator.

The yield was found to be little more than a third of that stated by these authors for the crude product. Allowing for the fact that our yield refers to the pure product, it is possible that there is a difference in xanthophyll content between the very young nettles we used as our material and the mature plant usually employed. The crystals were placed in an evacuated desiccator shaded from the light. They melted at 172° and gave the usual coloured solutions in organic solvents and answered the colour tests. Under the microscope the xanthophyll appeared as reddish yellow prisms.

Feeding test.

With the small quantity of crystalline xanthophyll available it was not possible to feed the substance to more than two albino rats. These animals were "run out" on the usual basal diet of this laboratory, vitamin D being supplied by direct irradiation for 10 minutes daily at a distance of 2 feet from the mercury vapour lamp. After their weights had become constant xanthophyll was administered at the rate of 3 mg. per head per day in the form of pellets. The pellets were made up with a small quantity of the basal mixture containing the correct dosage and were stored for the feeding period in a well-evacuated desiccator shaded from the light. These precautions are emphasised by the recent work of Schertz [1925], who finds that in the dry state at room temperature, xanthophyll is oxidised even more readily than carotene both in light and darkness. One pellet per day was fed by hand to each of the animals in advance of the basal ration, and very soon they were found to consume their dosage without objection. After a preliminary rise, both animals showed a marked decrease in weight which was not due to lack of appetite as the food intake was normal. Eventually both animals died and autopsies revealed the usual signs of vitamin A deficiency. Two control

animals fed 16 mg. of cod-liver oil in liquid paraffin gave a satisfactory growth response within 12 days, while both experimental animals died from vitamin A deficiency after 20 days' feeding with xanthophyll. It was therefore concluded that xanthophyll is ineffective in a daily dose of 3 mg. as a source of vitamin A.

SUMMARY.

Pure crystalline xanthophyll prepared from nettle leaves cannot be identical with vitamin A.

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REFERENCES.

- Drummond (1919). *Biochem. J.* **13**, 81.
Drummond and Coward (1920). *Biochem. J.* **14**, 668.
Drummond, Channon and Coward (1925). *Biochem. J.* **19**, 1065.
Jørgensen and Stiles (1917). *New Phytol.* Reprint No. 10.
Palmer and Kempster (1919). *J. Biol. Chem.* **39**, 299, 313.
Rosenheim and Drummond (1920). *Lancet*, i, 862.
Schertz (1925). *J. Agric. Res.* **30**, 575.
Stephenson (1920). *Biochem. J.* **14**, 715.