CXVIII. VITAMIN A FORMATION IN THE ETIOLATED WHEAT SHOOT.

By THOMAS MOORE.

From the Biochemical Laboratory, Cambridge.

(Received July 1st, 1927.)

As the result of extensive feeding experiments with green plant tissues, etiolated tissues, and seeds, Coward and Drummond [1921] established the superiority of green tissues as a source of vitamin A (not then differentiated from vitamin D). Very small amounts of vitamin were found in seeds and in etiolated tissues, which led to the inference that vitamin A formation in the growing plant was to be associated with the presence of chlorophyll. The vitamin was to be considered a product of photosynthesis.

A year later Wilson [1922] contradicted this view. Experiments were described in which the dried powdered stems of wheat grown to a height of 3 in. in the dark, and fed to rats at rates of 5 and 8 % of the basal ration in preventive and curative experiments respectively, were found to be as effective as the same amounts of dried powdered green stems in permitting growth on a diet deficient in vitamin A. Wilson concluded that light was unnecessary for the formation of the vitamin, and that if green shoots contained more vitamin A than similar etiolated shoots this was in all probability due to the acceleration of the physiological processes of the plant during photosynthesis.

To these conclusions Coward [1923] advanced the objection that no control experiments had been carried out to test the vitamin content of the seeds from which Wilson's etiolated shoots had been derived, a criticism which gained weight through the presence in his basal diet of 20 % of lard, which might have contained traces of vitamin just insufficient to support growth, but sufficient when supplemented by traces of vitamin from the seeds. Coward still maintained that light was necessary for the formation of the vitamin, but as the result of further experiments admitted that the presence of chlorophyll was not essential. Vitamin A formation could proceed in the absence of carbon dioxide or oxygen, but was prevented by the presence of chloroform.

By some authorities [Accessory Food Factors Committee, 1924; McCollum and Simmonds, 1925] the results of Coward and of Wilson have been considered contradictory, but while this is true of the conclusions reached the actual experiments seem to have been quite incomparable. Wilson's 5 and 8 % allowances of powdered stems may be calculated to have been derived from wheat equivalent to 18 and 29 % of the diet, and on a food intake of 10 g. 125 and 200 stems must have been fed daily to each rat. In reply Coward

quoted experiments in which the daily dose was limited to three etiolated shoots, or to the unsaponifiable matter from fifteen. Even at such a low level slight growth was observed in some cases.

Further experiments by Coward [1925] led to no change in her position, which was approved by Rosenheim and Drummond [1925]. Hess and Weinstock [1924], however, had shown that fresh etiolated wheat shoots, even when fed to rats at the rate of 10 g. daily, were ineffective in curing rickets, and it was obviously desirable to repeat the experiments with vitamin D, so intimately connected with sunlight, supplied by the irradiation of the diet, or some other means. By using a technique so modified and by increasing the daily allowance of shoots from three to five, Coward [1927] has recently detected the presence of appreciable quantities of vitamin A in etiolated wheat shoots, and has accordingly abandoned the view that light is necessary for the formation of the vitamin.

The experiments to be described, which were nearing completion when Coward's results were published, afford independent confirmation on this important point.

EXPERIMENTAL.

The wheat seeds used weighed on an average 20 to the g., and seem therefore to have been of a larger variety than those used by Wilson, which weighed 68 to the g. They were sprouted in moist sand contained in zinc trays, which were arranged in a wooden box. The box was painted black externally and the open side, by which the trays were inserted, was covered by a flap of four thicknesses of black calico, held in position by elastic bands. In the routine finally adopted all sowing, collection, and feeding was carried out after night-fall. Each tray accommodated seeds to provide shoots for 2 days' feeding. Sowings were accordingly performed on alternate days, the sand being strongly heated before each sowing to destroy bacteria. Sprouting was continued for 10 days, the shoots obtained varying somewhat in size in the different batches, but as a rule having stems about 4 in. long. In colour they were pale yellow.

Young rats, of weights from 42 to 60 g., were "run out" on the customary basal diet of this laboratory, 0.25 cc. of marmite extract and 0.2 cc. of lemon juice per rat per day supplying vitamins B and C respectively. Vitamin D was supplied by the administration of daily doses of 5 mg. of irradiated cholesterol from the points indicated on the accompanying growth curves. Out of a dozen rats the four weakest were used as positive controls (not shown), the remaining eight were given either etiolated shoots or seeds at the points indicated on the curves (Fig. 1), one bar corresponding to ten shoots or seeds, two bars to twenty, and three to thirty.

The four rats receiving seeds declined rapidly in weight, became rough-coated and emaciated, developed respiratory troubles and either died or were killed in dying condition. The rats receiving etiolated shoots, at first fed in a dark room in advance of the basal ration, remained comparatively smooth-

coated. Three rats (nos. 2, 3 and 4) fluctuated in weight, though gaining on the whole. The fourth (no. 1) showed a serious decline and developed respiratory troubles, possibly through delay in feeding the shoots. Unfortunately some of the animals did not regularly ingest all the shoots offered, usually nibbling the grain and discarding the stem and roots: it therefore became necessary to modify the routine. Thirty entire shoots were now allowed to each animal, the stems, roots, and grains being finely chopped and mixed intimately with the basal ration, which was fed after nightfall and consumed before morning. In addition, the rats were irradiated before a mercury arc lamp for 10 minutes daily.

Fig. 1. Formation of vitamin A in the etiolated wheat shoot.

× = Feeding of irradiated cholesterol commenced.

|, ||, ||| = 10, 20 or 30 seeds or etiolated shoots fed *per diem*.

K = killed.

D = died.

G = 30 green shoots fed *per diem*.

C.L.O. = 2 drops of cod-liver oil replaced shoots.

As the result of such treatment growth was restored in all four cases, demonstrating beyond doubt the activity of the etiolated wheat shoots in repairing deficiency of vitamin A. At the time of the adoption of the revised routine, however, only two animals of the control group remained alive. Two more rats were therefore placed on the basal diet, receiving in addition an allowance of thirty wheat seeds each per day and direct irradiation from the commencement of the experiment. After the normal period they declined and died, one displaying severe symptoms of xerophthalmia.

DISCUSSION.

Since feeding was conducted after nightfall the shoots were exposed only to artificial illumination before ingestion by the rats. It seems improbable that illumination of such feeble intensity should cause a formation of the vitamin. The rats themselves, however, were not kept in the dark, and a loophole for photosynthetic action remains open if the formation of the vitamin, as in the case of vitamin D, be pictured to take place in the animal on exposure to diffuse daylight or to mercury arc illumination. Further experiments are contemplated to clear up this point. For the present there is no reason to believe that vitamin A is not produced by the plant in the dark. If it is not formed under such conditions a precursor must at least make its appearance, since wheat seeds fed under similar conditions of illumination were found to be ineffective.

From the growth curves it will be seen that for a period of several weeks all the rats receiving etiolated shoots grew at an approximately normal rate. In the case of rat no. 1 (male) such growth was continued without serious check for about four months, a weight of 172 g. being attained 115 days after the addition of shoots to the diet. The remaining rats (nos. 2, 3 and 4 (females)) after a time ceased to grow, marked plateau periods in the growth curves being attained at body weights of about 130 g. The cause of this stoppage is not known. Although no acute symptoms, beyond occasional sneezing, were observed, all the four rats were obviously in an imperfect state of nutrition, rib bones being unduly prominent, coats slightly rough, backs slightly arched and muscular tone poor. A partial insufficiency of the vitamin might well be suspected, but shoots were fed at six times the minimum determined by Coward; moreover the replacement of etiolated shoots successively by green shoots and by cod-liver oil in the diets of two of the rats, while promoting slight growth, did not notably improve their condition above that of the others. Whatever the cause of such imperfect nutrition may have been, however, it would appear certain that the etiolated shoots, directly or indirectly, provided an active source of vitamin A, since the rats could certainly not have survived the control animals for several months without any of the characteristic signs of deficiency in the continued absence of that vitamin.

In the present experiments, it should be observed, shoots at an advanced stage of development were employed. In experiments with wheat and corn

seeds germinated for shorter periods (20–48 hours), Coward [1927], Harrow and Krasnow [1922, 1924], and Karshan, Harrow and Krasnow [1927] have failed to establish any increase in vitamin A content above that of ungerminated seeds, and it has been suggested that the vitamin is not synthesised under such conditions. In Coward's experiments, however, only five germinated wheat seeds were allowed per rat per day, a level dangerously low in view of past experiences at a three-shoot dosage, while in those of the American workers vitamin D was not supplied. Such evidence, therefore, while of obvious importance from an economic standpoint, cannot afford an adequate basis for the absolute denial of the formation of traces of vitamin A, and for the present the question should be left open. Stepp [1925], it may be mentioned, has reported the synthesis of traces of vitamin A in wheat seeds germinated for 4 days, but as no precautions were taken to exclude the action of light this finding cannot be considered relevant to the problem under discussion.

SUMMARY.

Fresh etiolated wheat shoots, 10 days old, fed to rats receiving a diet deficient in vitamin A, but adequate in vitamin D, at the rate of thirty shoots per day (equivalent to 1.5 g. of dry wheat) prevented death and restored growth. Seeds fed at the same rate were ineffective.

This result is in agreement with statements by Wilson [1922] and Coward [1927] that vitamin A may be formed in etiolated tissues. In the present experiments the feeding of shoots was continued for some months after the death of the control animals. The final condition of the animals was fair, although they appeared somewhat lean and rough-coated when compared with similar rats receiving a liberal diet.

My thanks are due to Sir F. G. Hopkins for support and criticism.

REFERENCES.

- Accessory Food Factor Committee (1924). *Med. Res. Council. Special Report Series*. No. 3B. Revised, 27.
 Coward (1923). *Biochem. J.* **17**, 134.
 — (1925). *Biochem. J.* **19**, 500.
 — (1927). *J. Biol. Chem.* **72**, 798.
 Coward and Drummond (1921). *Biochem. J.* **15**, 530.
 Harrow and Krasnow (1922). *J. Metab. Res.* **2**, 401.
 — (1924). *Proc. Soc. Exp. Biol. Med.* **21**, 232.
 Hess and Weinstock (1924). *J. Biol. Chem.* **62**, 310.
 Karshan, Harrow and Krasnow (1927). *Proc. Soc. Exp. Biol. Med.* **24**, 765.
 McCollum and Simmonds (1925). *The newer knowledge of nutrition*. 3rd ed. p. 314.
 Rosenheim and Drummond (1925). *Biochem. J.* **19**, 751.
 Stepp (1925). *Z. Biol.* **83**, 94.
 Wilson (1922). *J. Biol. Chem.* **51**, 455.