CXXXV. VITAMIN A FORMATION. THE FEEDING OF ETIOLATED WHEAT SHOOTS TO RATS KEPT IN DARKNESS.

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(Report to the Medical Research Council.)

(Received June 30th, 1928.)

ALTHOUGH an accumulation of evidence [Coward and Drummond, 1921; Coward, 1923, 1, 1925; Harrow and Krasnow, 1922, 1924; Karshan, Harrow and Krasnow, 1927; Dye, Medlock and Crist, 1927; Heller, 1928] has pointed to some relation between photosynthesis and abundance of vitamin A in plant tissues, ample proof has been afforded that the feeding of etiolated wheat shoots grown in the dark may effectively repair a deficiency of the vitamin [Wilson, 1922; Coward, 1927; Moore, 1927]. Such evidence would suggest that the action of light is not an essential for the formation of the vitamin, serving only to accelerate processes which occur spontaneously in the dark, but before this conclusion can be drawn it is necessary to decide whether such short periods of illumination (of the shoots or the experimental animal) as are admitted in the normal technique of rat feeding may have played any part in the synthesis.

It may be allowed at once that should the appearance of chlorophyll be adopted as the sole criterion of photosynthesis further research on the point would be unnecessary, since with a hungry rat the shoots need remain uneaten and exposed for only a short period, but such a criterion cannot well be justified. Experience has shown that a very short exposure to ultra-violet irradiation will suffice for the production of vitamin D from ergosterol, and it is conceivable that equally short exposures to visible light might effect the synthesis of vitamin A in the etiolated plant before there is time for noticeable "greening" to take place. Apart from action on the plant tissues, an additional possibility, as in the case of the production of vitamin D, is the action of light upon the animal *in vivo*, after the absorption of a provitamin derived from the shoots.

In control experiments it has been found that ungerminated wheat seeds, fed to rats in numbers equal to effective doses of etiolated shoots, do not noticeably delay the decline and death of the animals, even when no precautions are taken to shield the seeds and animals from light. During growth in the dark, therefore, at least one step in the synthesis of the vitamin is accomplished. It remains to be decided whether the vitamin is completely

synthesised in this one step or whether a precursor is first formed by non-photosynthetic processes and subsequently activated by very short periods of illumination.

Rosenheim and Drummond [1920] once suggested that vitamin A might be produced by the action of light on a pigment of the carotinoid type, and it has been shown by Coward [1923, 2] that during the growth of the etiolated wheat shoot there is a considerable synthesis of the pigment xanthophyll. Recently [Rosenheim, 1927] the trend of speculation has been centred more on ergosterol than on the carotinoids as a precursor of vitamin A, but the possibility of any mechanism of a similar "two stage" nature emphasises the need of caution before it is finally assumed that light plays no part in the production of the vitamin.

The experiments to be described were planned to test the vitamin A content of etiolated wheat shoots under "dark room" conditions, a minimum intensity of red light being admitted to allow the necessary manipulations of the shoots and animals.

EXPERIMENTAL.

The experiments were conducted in a darkened room, warmed by a covered gas fire and ventilated by shafts, which were arranged vertically and blackened internally to prevent reflection of light. Entrance to the room could be effected by means of a double door. For constant illumination a small ruby lantern was provided; the animals and their food could barely be distinguished by its light when the eye had become adjusted to darkness. For the purpose of collecting shoots and preparing diet somewhat brighter illumination was required, and since electric light was not available, light was admitted when necessary through a small ruby glass window protected by a shutter. The shoots were grown, as in the previous experiment [Moore, 1927], upon moist sand for a period of 10 days. To avoid unnecessary exposure to light from the ruby window the trays were covered with a screen of black calico.

For testing the activity of the shoots two groups (A and B) of four young piebald rats each and two groups (C and D) of four young Wistar albino rats each were used. To all four groups a standard basal diet prepared by the British Drug Houses was supplied, supplemented in all cases by an allowance of 0.75 cc. of marmite extract per animal daily. In the case of groups B and C, used to test the main question of the vitamin A content of the shoots, and of group A, the negative control group receiving seeds, the diet was further supplemented by an allowance of one drop of "radiostol" (a preparation of 0.1 % of irradiated ergosterol in arachis oil) per animal daily. Group D was used to test the effect of the omission of vitamin D from the diet when etiolated shoots were supplied from the commencement of the experiment, and the "radiostol" was accordingly omitted.

As shown by the accompanying growth curves all four animals in Group A declined in weight after a period of 7 weeks on the experimental diet, signs

of xerophthalmia being evident. Upon the addition to the diet of 30 wheat seeds per animal daily no response was obtained, and the animals died. In the case of groups B and C, which received respectively 30 and 15 etiolated wheat shoots per animal daily after growth had slackened, a slow resumption

Fig. 1. Growth curves.

in growth was obtained in six animals. One of the remaining two animals (Group B, No. 4) after a preliminary response during several weeks declined slowly and died from intestinal stoppage; the other (Group C, No. 4) had

probably already reached a point of rapid decline in which the feeding of a source of such low activity as the etiolated shoot was ineffective. In the case of Group D, 15 shoots per rat daily were allowed from the commencement of the experiment, with a view to deciding whether vitamin D could be made to become the limiting factor in growth when vitamin A was supplied by etiolated shoots, and whether the "radiostol" supplied in the preceding experiments had been an essential or merely precautionary measure. Only one rat showed an early decline, and death was not prevented by the administration of "radiostol" in large doses. The remaining rats attained weights at which some slackening in growth might well have been anticipated from the behaviour of rats receiving both shoots and "radiostol," and the experiment was discontinued.

DISCUSSION.

Since the results obtained under "dark room" conditions differed in no way from those obtained under ordinary conditions of illumination, it is obvious that should light be concerned in the synthesis of the vitamin the exposures necessary are extremely short, and that red light is effective. A strong temptation must exist to assume that the synthesis of the vitamin is independent of light, but this must be qualified by a consideration of the extreme sensitivity of etiolated plant tissues to diffuse light. To illustrate the point it may be mentioned that while the exposures to red light permitted in the experiment did not cause noticeable "greening" of the shoots, inadvertent exposure to this light for some hours of a trial batch of shoots, before the commencement of the actual experiment, resulted in quite definite greening. If the complicated chlorophyll molecule can be synthesised after such short and diffuse exposure it must remain a possibility, though no more than a possibility, that vitamin A may be synthesised during even shorter exposures.

In general the trend of the experiments has been to confirm the view that the etiolated wheat shoot is a definite but unsatisfactory source of vitamin A. While xerophthalmia is cured and a slow resumption in growth is promoted, rats receiving etiolated shoots are often lean and rough coated, and particularly liable to sneezing and lung troubles. This may probably be attributed simply to the low content of vitamin in the shoots, but it must be remarked that even when comparatively large doses of shoots are supplied, growth and condition seem to be no better than with smaller doses. Thus Coward [1927] has found that five etiolated wheat shoots are sufficient to procure slow growth; the effect of feeding 15 or 30 shoots would not appear to produce better results. In our experiments animals receiving 15 shoots daily grew as well as, or better than, rats receiving 30 shoots, and it is possible that the inclusion of large amounts of moist vegetable tissue in the diet of rats is harmful. This possibility was supported by the finding of large amounts of undigested fibres in the gut of the rat (Group B, No. 4) which died from some intestinal disturbance, but no systematic enquiry into the matter has been made.

SUMMARY.

Etiolated wheat shoots tested upon rats under conditions involving the minimum of red light illumination consistent with the feeding and handling of the animals were found to be effective as a source of vitamin A, supporting the conclusion that light is not essential during any stage of the formation of the vitamin from the seed.

My sincere thanks are due to Dr L. J. Harris for valuable advice and criticism, and to Mr Alfred Ward for technical assistance.

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