or less in length. These cells retain their meristematic character while the neighboring cells continue in the process of differentiation forming the body of the mature leaf. In actively functioning leaves 8 to 10 centimeters long this group of meristem cells may show a more or less distinct differentiation of root and shoot primordia. The writer has chosen to call these meristematic cell masses "foliar embryos" rather than "foliar buds" or "epiphyllous buds," since root and shoot develop simultaneously from them and may even be present in a primordial condition on a large, normal, attached leaf. Only a slight stimulus of the proper sort is required to cause the foliar embryos to continue their development into a new plant. Under normal cultural conditions such development does not occur on attached leaves yet it would seem that to refer to the roots and shoots produced as "adventitious buds and roots" and to include them under the term "regeneration" would be to employ vague or even incorrect terminology. A careful study of the various phenomena commonly grouped under "regeneration" makes it clear that the task of defining and limiting this term is difficult, but the writer suggests that, in cases where a preformed meristem exists which is definite and localized in position and which merely continues development due to some stimulus, the term "regeneration" is hardly applicable.

The existence of vegetative patches or centers upon the leaves of *Bryophyllum* is by no means a recently discovered fact for Goebel[4] refers to them, and Kerner and Oliver[5] also describe them in a superficial way. Yet few facts seem to exist concerning their structure and developmental history. Lund and Bush[6] diagram a section through the foliar embryo but otherwise make no statements regarding its structure and development except a reference to the work of Beals.[7] To the writer's knowledge this last named work is the only available histological study of the development of the plantlets upon the leaves of *Bryophyllum*. Beals draws the conclusion that certain phloem cells of the leaf assume meristematic activity and build up the tissue of the new plant. No mention was made of the dormant foliar embryo which exists even in very young leaves and which in older leaves is evident to the most casual observer. From Beals' paper it is evident that she was experimenting with fairly mature leaves.

While no attempt has been made in the present study to determine the physiological causal factors involved in the awakening of these foliar embryos it is obvious that such study must take account of their presence and structure. There is no space in the present brief note to give details of the writer's study and findings but it is hoped that they may be published *in extenso* at a later date.

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[4] K. Goebel, "Organography of Plants," I, 42, 1900.

[5] Kerner and Oliver, "Natural History of Plants," II, 40, 1903.

[6] Lund and Bush, *Plant Physiology*, 5; 491, October, 1930.

[7] C. M. Beals, *Ann.* Miss. Bot. Garden, 10: 369, 1923.

A NEW PLANT SOURCE OF VITAMIN A ACTIVITY[1]

THE recent interest in carotin and its physiological action has encouraged the examination of various plants as a source of this material. It occurred to the writers that a further investigation of the coloring matter annatto, obtained from the seeds of the plant *Bixa orellana,* might be of interest.

At least two pigments have been isolated from the seeds of this plant. Bixin, the better known of the two, has been much studied and we owe a knowledge of many of its chemical and physical properties to the researches of Marchlewski[2] in 1907.

Since that time many papers have appeared dealing with its chemical structure and properties, but Euler and Euler[3] in 1929 were apparently the first to test its physiological activity, which they reported as being negative. Palmer[4] in his monograph states that bixin does not belong to the group of carotinoid pigments, and therefore might reasonably be expected to be inactive in this respect. Palmer does not mention the less known pigment orellin, which accompanies bixin, and it is the latter material that is the basis of the present investigation.

If the crude red powder (annatto) obtained from fresh seeds of Bixa Orellana is extracted with cold 80 to 90 per cent. alcohol, a deep reddish-brown solution results, which on evaporation leaves a dark-colored, sticky, resinous material. It is in this fraction (practically bixin-free) that the vitamin A activity resides. When an alcoholic solution of this resinous material containing orellin is fed to rats on a vitamin A-free diet, at such a level that they receive 3 mg of dissolved solids per day, their rate of growth corresponds to that recommended by Sherman[5] in his quantitative estimation of this vitamin. So far as the semi-quantitative results show at present, the seeds yield 2 per cent. of this active material. This places

[1] From the School of Tropical Medicine of the University of Porto Rico under the auspices of Columbia University, San Juan, Porto Rico. This research was made possible by a grant from the Rockefeller Foundation.

[2] L. Marchlewski, *Biochem. Z.*, 3, 286, 1907.

[3] Beth v. Euler and Hans v. Euler, *Helv. Chem. Acta.*, 12, 278, 1929.

[4] L. S. Palmer, "Carotinoids and Related Pigments." Chem. Monograph Series, 1st ed., 22, 1922.

[5] H. C. Sherman and H. E. Munsell, *Jour. Amer. Chem. Soc.*, 47, 1639, 1925.

annatto among the richest vegetable sources of vitamin A thus far reported.

It is unknown at this stage of the investigation whether we are dealing with vitamin A as such, carotin or some related pigment, or a new substance which can function as vitamin A in the animal body.

A preliminary experiment of feeding bixin obtained from annatto that had had the active resinous coloring material removed by alcoholic extractions confirmed Euler's finding in that it did not cause growth in rats on an A-free diet. There is some indication that bixin may exert a toxic action on the organism.

Further work is in progress on this interesting development.

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A NOTE ON THE EFFECT OF ULTRA-VIOLET LIGHT ON THE VITAMIN A OF BUTTER

Zilva[1] has shown that vitamin A is destroyed by ultra-violet light when exposed in air but not in a carbon dioxide atmosphere. Spinka[2] found that ultra-violet radiations did not destroy vitamin A, but that toxic materials were formed which were sufficient to cause death in rats. His animals succumbed before the controls on a vitamin A free diet developed the typical symptoms of vitamin A deficiency.

The fact that radiations other than ultra-violet light[3] destroy the vitamin A of butter suggested that the mechanism might be of a photochemical nature.

Butter fat was exposed in large flat dishes to the rays of the quartz mercury arc at a distance of 15 cm. for 6 hours. At the end of 3 hours all the color of the material had disappeared. Another portion of the fat was exposed under similar conditions in an atmosphere of nitrogen which had previously been purified to free it from oxygen. No change in color was observed. These materials and the untreated butter fat were fed to rats depleted of their vitamin A stores, as follows: (1) The untreated butter fat; (2) butter fat exposed to ultra-violet light in air; (3) butter fat exposed to ultra-violet light in a nitrogen atmosphere; (4) the untreated material mixed in equal parts with the butter fat exposed to ultra-violet light in the air. Control animals received no source of vitamin A. The results are presented in Fig. 1.

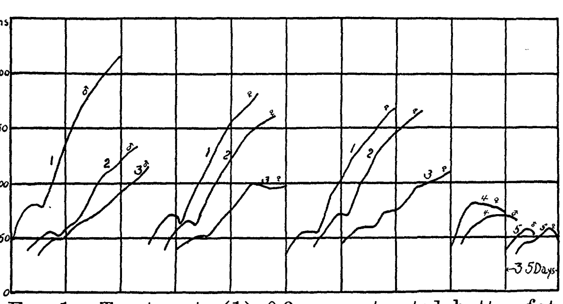

Fig. 1. Treatment. (1) 0.3 gm untreated butter fat, daily. (2) 0.3 gm butter fat irradiated in nitrogen atmosphere, daily. (3) 0.3 gm untreated butter fat plus 0.3 gm butter fat irradiated in air, daily. (4) 0.3 gm butter fat irradiated in air, daily. (5) No source of vitamin A.

Results

Practically complete destruction of vitamin A potency and loss of color in butter fat was obtained when the material was exposed in air. The material exposed in a nitrogen atmosphere did not fade. Its antiophthalmic properties were not reduced in any detectable degree, although some reduction in its growth-promoting power was found.

When butter fat exposed in the air was mixed with the untreated material a definite slowing of growth was obtained as compared to that produced by untreated butter fat fed in an equivalent amount.

Negative controls and animals receiving butter fat exposed in the air succumbed at about the same time. Ophthalmia developed in both groups.

These experiments indicate that the change that takes place in vitamin A potency when butter fat fades is not due to a direct effect of ultra-violet light. Oxidation, indirectly produced by ultra-violet radiations, is at least one mode of destruction of the vitamin A of butter fat. It appears that irradiation in air and to some extent in a nitrogen atmosphere produces a principle that retards growth of rats. This principle was not of sufficient strength to cause death before the onset of vitamin A deficiency symptoms.

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[1] S. S. Zilva, *Biochem. Jour.*, 13: 164, 1919: 14, 740, 1920.

[2] J. Spinka, *Biochem. Ztschr.*, 153, 197, 1924.

[3] A. G. Hogan, C. L. Shrewsbury and J. F. Breckenridge, *Jour. Biol. Chem.*, 87, p. xlii, 1930.

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