the rubber tubing, making a simple rubber joint. It is best to daub rubber cement or shellac on the glass tubing before making the connection.

A string is now tied to two of the wire loops, and another string is fastened onto the glass tubes protruding from the rubber joint. Both of these connecting strings are tied so that there is plenty of slack in them. A hand cord is attached somewhere along the length of each connecting string. The two hand cords serve to open and close the valves as well as raise and lower the entire instrument. A lead weight is suspended from the third wire loop which is fixed on the opposite side of the tube from the other loops and at one end. This weight serves to sink the apparatus as well as to slant it so that water can come in the lowest side and force the air from the higher end.

The apparatus is used in the following manner. The weight of the instrument is suspended from hand cord No. 1 (Fig. A) which is attached to the ends of the glass tubing. This action kinks the rubber connections and forms a perfectly air and water-tight valve. With the weight of the instrument supported from cord No. 1 the apparatus is lowered into the water. During this phase the No. 2 cord is paid out very loosely. When the selected depth has been reached the weight is shifted to cord No. 2 and cord No. 1 is loosened. This action allows the rubber connections to straighten out and the valve to open. When the body of the apparatus has been filled the weight is again transferred to cord No. 1, which closes the valves. With the valves closed the instrument is pulled to the surface. The rubber connections may be kept permanently closed by tying strings around the connecting pieces.

In bacteriological work a number of such instruments may be made and sterilized in the autoclave, provided the binding of the wire loops has been fixed with waterproof Valspar varnish. To remove the contents of the tube in a sterile manner the rubber valves may be cut close to the glass tubing.

Types of work other than bacteriology may require apparatus with larger openings. Apparatus have been made at this university with openings up to an inch in diameter with but a few minor changes in the shapes of the glass pieces forming the valves and the use of a double system of rubber connections.

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SPECIAL ARTICLES

REGENERATION IN BRYOPHYLLUM

WHILE *Bryophyllum calycinum* has been repeatedly used in physiological investigations of the phenomenon of "regeneration," a developmental and histological study of the foliar organs of this plant gives rise to a grave doubt whether in this instance regeneration really occurs. The worker is dealing with leaves possessing latent meristems in their notches which quickly form both root and shoot systems when the proper stimulus is applied. Even so able an investigator as Jacques Loeb[1] disregards entirely the presence of these foliar meristems or embryos and L. W. Sharp[2] refers to *Bryophyllum* as a case in which dedifferentiation of cells takes place in the formation of plantlets upon the leaves. A study of these problems being carried on by the writer reveals that too little attention has been paid to the anatomy and developmental history of the leaves of *Bryophyllum* and that physiological studies must take account of these facts if they are to interpret correctly the processes involved in so-called "regeneration."

The question immediately arises as to what the phenomenon of regeneration really involves. Are we to consider as regeneration only such phenomena as the reformation of a tail in the case of certain snakes, the replacement of an eye-like structure in the case of *Cambarus*, or the reformation of a growing point on the shoot axis of a plant? Or shall we include within this category the development of latent buds in the willow, of axillary buds (in many plants) which in the normal course of events never develop, and finally the development of the foliar embryos in the leaves of *Bryophyllum*? All these examples have been lumped together under the term "regeneration" by various workers and since the wide differences existing among them are obvious, the situation is a rather unhappy one.

Particularly is this the case when development of plantlets upon the leaves of *Begonia* is termed "regeneration" and the same term applied to plantlet development upon the leaves of *Bryophyllum*. Hartsema[3] has clearly shown that in the case of *Begonia* there is an actual dedifferentiation of certain cells of the epidermis and an assumption by them of meristematic characters which builds up a new plant. Work of the writer shows that in the case of *Bryophyllum* a group of meristem cells is very early segregated in the notch of the leaf even when it is 2 mm

[1] Jacques Loeb, "Regeneration," 1924.
[2] L. W. Sharp, "Introduction to Cytology," 1926.
[3] A. M. Hartsema, Extrait du Recueil des Travaux botaniques néerlandais, Vol. 23, 1926.

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.