

he always responded to the limit of his ability. In 1902 he went as far as Russia. "We were given a magnificent reception by the Mayor of Moscow, Prince Galitzin, and conducted through all the prisons and institutes of the city. He then invited all the guests to a supper, when the Tsar's health was drunk on champagne. To the horror of Frau von Wolfring, I did not join in drinking the toast, since there was no water on the table! . . . Moscow was at that time a curious mixture of barbarism and culture, with striking contrasts between wealth and poverty, education and ignorance, integrity and corruption, feasting and starvation. And everywhere society was fermenting under the surface."

In 1910, Forel suffered a great blow in the death of his oldest son Eduard, who had just passed his medical examination, and was thought of as the one to continue and develop the great traditions of his father's work. Two years later, just as he was preparing to go on a long voyage to tropical Asia, Forel suffered a stroke, which seemed at first to threaten total disability. He partially recovered, and went on with his studies of ants. The great war of 1914-1919 stirred him up to new activities, in the cause of peace and better social conditions. Forel's matured political faith is eloquently stated by him as follows (written in 1920):

"Socialism is now inevitable, and the social work of 1919 and the following years was, as we see, foretold

by Lange. A strong capitalistic, monarchistic and militaristic reaction could only lead to international anarchy and fresh wars, which might indeed delay the final, international social reform by a few decades, but could never, never prevent its final victory. One must be really hidebound and crazy not to see this today, after such infinite ruin, after such an aimless shedding of blood. The modern technique of intercourse, almost unknown a century ago, has to-day made the World Federation of Peoples not only possible, but inevitable. But this will *quite automatically* abolish wars between states, which without it have grown constantly more extensive. Modern warfare is war waged *ad absurdum*. This, in a few words, is my *political and social testament*. Predatory, egoistic and hypocritical though human nature may be in itself by inheritance, yet it can be tamed from childhood upwards *by social education*. My perception of this I owe in the first place to Pierre Huber—that is, to the study of the ants; then to the study of the dead and living brain of men and animals; then to psychiatry, hypnotism, psychotherapy and medical psychology; further, to Darwin and Semon, and at last, but not least, to my dear wife, and to abstinence from alcohol, to speak only of persons and studies and activities which have had the profoundest influence on my career."

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SPECIAL ARTICLES

PURIFICATION OF TRAUMATIN, A PLANT WOUND HORMONE

ONE of the classical problems of botany is that presented by the "wound hormone," a concept first proposed by Wiesner¹ and later elaborated upon by Haberlandt² and others.³ From the experimental evidence which has accumulated it may safely be concluded that when a plant tissue is injured, a substance or substances are produced which are capable, under suitable circumstances, of inducing renewed growth and division of other, uninjured, mature parenchymatous cells. That this hormone is necessary for the successful cultivation *in vitro* of some plant tissues has also been demonstrated.⁴ It may, in addition, play a rôle in such processes as the healing of wounds, callus formation and adventive embryony.² In investigations which will be reported in detail elsewhere the present authors have undertaken a

chemical and physiological study of such a "wound hormone."

A quantitative physiological assay for wound hormone was developed upon the basis of Wehnelt's⁵ discovery that the parenchymatous tissue lining the seed chambers of immature string bean pods reacts to the application of a drop of tissue extract with the formation of a cylindrical "neoplasm" or intumescence. The new growth is the product of simultaneous cell division and cell enlargement. As a measure of activity the present authors have used (a) the concentration of wound hormone needed to produce an intumescence of a definite, relatively large size, and (b) the minimum concentration of active extract which gives a measurable response.

It has been found that the "bean test" is specific for the wound hormone in question. Other substances, such as hetero-auxin, pantothenic acid, etc., cause a response only if they are used in toxic concentrations which cause injury to, and the liberation of hormone from, the test cells themselves.

Using this quantitative assay to work out and to

⁵ B. Wehnelt, *Jahrb. wiss. Bot.*, 66: 773, 1927.

¹ J. Wiesner, "Elementarstruktur," Wien, 1892.

² G. Haberlandt, *Beitr. allg. Bot.*, 2: 1, 1921; *Biol. Zent.*, 42: 145, 1922.

³ H. Reiche, *Zeit. f. Bot.*, 16: 241, 1924; A. Wilhelm, *Jahrb. wiss. Bot.*, 72: 203, 1930.

⁴ J. Bonner, *Proc. Nat. Acad. Sci.*, 22: 476, 1936.

check each step, a procedure for the purification of the wound hormone has been developed. This procedure involves: (1) Extraction from dried bean pods with alcohol; (2) adsorption of the active fraction on charcoal and elution with pyridine; (3) fractional precipitation of impurities from pyridine with ether; (4) extraction of the hormone with ethyl acetate; (5) conversion of the active principle to its barium salt; (6) precipitation of the active principle with mercuric acetate; (7) extraction of the hormone with acetone; (8) formation of the methyl ester; (9) fractional distillation of the ester under high vacuum and subsequent hydrolysis of the redistilled ester to regenerate the active free acid.

Products of constant activity and identical composition have been obtained from three separate large-scale extractions. The ester product of 9 seems to be not far from pure, although it has not yet yielded a single crystalline derivative. A few of its properties will therefore be reported here in a preliminary form. The products of 7 and of 9 (hydrolyzed) possess the same activity in the "bean test" and give a measurable response at a dilution of 1:100,000. The "free acid" of 7 is a water soluble, extremely hygroscopic, colored, amorphous solid. The ester is a yellow oil, readily soluble in ether, chloroform, etc., but nearly insoluble in water. Its analysis and molecular weight agree approximately with the formula $C_{11}H_{17}O_4N$, including one methyl group introduced during the esterification. Other evidence indicates that hydroxyl groups are absent and that a second carboxyl group is present as a betaine, inner amide or a lactone, of which the first seems the most likely. The wound hormone obtained here is thus clearly different from that concentrated by Umrath and Soltys⁶ from alfalfa. Only small amounts of ester have as yet been available, and further extractions upon a larger scale are necessary to firmly establish its purity and structure.

The authors propose the name "traumatin" for this principle which is active in the bean test. This name seems particularly appropriate in view of the "wound hormone" historical background of the substance which has been isolated.

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ALGAE AND GROWTH-SUBSTANCES

THIS is a preliminary report of the effect of certain growth-substances on some unicellular algae.

⁶ K. Umrath and A. Soltys, *Jahrb. wiss. Bot.*, 84: 276, 1936.

Since growth-substances affect plant cells in the embryonic state it was deemed desirable to use unicellular algae which reproduce by means of autospores. Young autospores are highly sensitive and respond quickly to stimuli after the manner of embryonic cells. The three algae used were *Chlorella vulgaris*, *Chlorella pyrenoidosa* and *Oocystis* sp.

A large number of synthetics were employed in growing pure cultures of algae. It was found desirable to exclude sugars and limit the source of carbon to carbon dioxide dissolved in the culture media and carbon in the monobasic acids used.

The synthetic that gave the most satisfactory results had the following formula: potassium nitrate, 1 gr., calcium sulphate, 0.5 gr., magnesium sulphate, 0.5 gr., ferrous phosphate, 0.10 gr., tri-calcium phosphate, 0.25 gr., distilled water 1,000 cc.

It is very important that the correct pH be maintained in all cultures of algae treated with growth-substances. If the acidity or the alkalinity is high the algae are seriously disturbed and lethal action will take place. After many tests it was found that the optimum pH was 5.6-6.5. If the pH were not well buffered at this optimum the readings of the effect of the growth-substances were definitely modified.

The four monobasic acids used as growth-substances were: a-naphthaleneacetic acid, indole-3-acetic acid, indole-3-propionic acid and phenylacetic acid. In the early experiments water solutions of the acids were employed. In later experiments the growth-substances were dissolved in 95 per cent. alcohol—10 mg per cc of alcohol. The alcoholic solution remains unchanged for an indefinite period, whereas water solutions change and give variable results. After adding the alcoholic solution of the growth-substance in various concentrations to test-tubes containing 10 cc of the synthetic the alcohol was removed by autoclaving for 30 minutes at 15 pounds pressure.

The growth-substances were used in concentrations from 1-10,000 to 1-3,000,000. Concentrations from 1-10,000 up to 1-50,000 were lethal for the three species of algae studied. The lower concentrations (1-100,000 up to 1-3,000,000) were positive in their stimulating effect on all cultures compared with the controls. *Chlorella vulgaris* gave affirmative results in from 60 to 72 hours, *Chlorella pyrenoidosa* in from 96 to 120 hours and *Oocystis* sp. in from 150 to 170 hours.

There was some evidence that growth-substances accelerated the rate of cell reproduction and increased the size of cells in the algae studied.

The study of growth-substances on plant tissue has been largely confined to multicellular plants. In those cases there is inevitable masking of the reaction of individual cells to growth substances by neighboring cells. While this difficulty may be obviated, in a mea-