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 Soltys[6] 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-

sure, by microchemical technique, it seemed particularly desirable to use unicellular plants in one-celled cultures. Hence unicellular algae were employed in mass cultures in test-tubes, in one-celled cultures in Van Tiegham cells and on agar plates.

A series of quantitative measurements is being compiled and will be published later.

The tests reported in this paper were begun in September, 1935, and have been carried on continuously since that date in the botanical laboratories of the University of Wisconsin. Assistance has been received from the Wisconsin Alumni Research Foundation. I am indebted to Dr. W. P. Zimmerman for the growth-substances used and for helpful suggestions relative to their use with algae.

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TEMPERATURE AND THE GROWTH OF HAIR

CASUAL observation of the variation in the growth of facial hair at different seasons of the year suggested its measurement. An experiment was planned, involving the measurement of the hair shaved from the same part of the face at approximately the same hour and with the same technique every day for one year (the subject, P. E., a florid male, aet. 59).

The crop harvested with one stroke of a straight razor from an area of about one square inch on the right cheek immediately in front of the ear, was washed free from soap, dried and mounted. On each slide selected for measurement one hundred hairs chosen at random were measured with an ocular micrometer. From each month's samples ten were selected for measurement; usually, the first, second and third; the eleventh, twelfth and thirteenth; the twenty-first, twenty-second, twenty-third and twenty-fourth. Each daily value was linked with the average temperature of the preceding day, as furnished by the U. S. Weather Bureau.

Table I gives the average rate of growth and the mean temperature.

TABLE I

Month	Mean temperature	Measured growth
January	58°	.305 mm.
February	54°	.386 "
March	61°	.404 "
April	70°	.458 "
May	74°	.464 "
June	81°	.516 "
July	83°	.533 "
August	82°	.538 "
September	79°	.545 "
October	73°	.533 "
November	64°	.495 "
December	60°	.375 "

A "scatter diagram" constructed from the individual daily measurements shows a very interesting break in the regression line at about 65° Fahrenheit, a temperature which ordinarily calls for heating of homes and office buildings.

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VITAMIN B_1 CRAVING IN RATS[1]

WELL-AUTHENTICATED instances of excessive appetite or craving for special food stuffs are not numerous. We do know that some animals show a marked craving for common salt; other animals show an increased craving for phosphorus, expressed in a desire to eat bones; and some animals, including humans, may have a special appetite for calcium as shown by their ingestion of plaster and chalk. These cravings apparently have their origin in deficiencies produced either by a decreased intake of these minerals, or by an altered mineral metabolism in the case of sodium and calcium craving, dependent on changes in the adrenal[2] and parathyroid[3] glands, respectively.

Another and possibly more powerful craving may now be added to this list: the craving for vitamin B_1. In earlier experiments it was found that normal as well as vitamin B deficient rats show a great appetite for dried baker's yeast, which contains large quantities of vitamin B in addition to many other substances.[4] By virtue of the voluntarily increased ingestion of yeast the animal quickly lost all symptoms of vitamin B deficiency. This is in agreement with observations made by Harris, Clay, Hargreaves and Ward.[5] However, contrary to the results of Harris, *et al.*, we have found that rats show an overwhelming appetite for vitamin B in pure crystalline form, either as B_1 (betaxin or betalin), or Riboflavin. Vitamin B_1 was given in the form of an aqueous solution of synthetic thiamine chloride (betaxin, Winthrop Chemical Company, and betalin, Eli Lilly Company) in graduated inverted bottles. One vitamin deficient rat drank 11 cc, or 5,500 international units, in less than half an hour; another rat drank 29 cc, or 14,500 international units, in 24 hours. The odor of the vitamin as well as its taste arouses great interest. This is shown by the fact that the rats found the bottles at once, even when as many as twelve other containers filled with different foods or solutions were present in the cage at the same

[1] From the Psychobiological Laboratory, Henry Phipps Psychiatric Clinic and the Harriet Lane Home for Children, Johns Hopkins Hospital.

[2] C. P. Richter, *Endocrinology*, 20: 657–666, 1936.

[3] C. P. Richter and J. F. Eckert, *Endocrinology*, 21: 50–54, 1937.

[4] C. P. Richter, L. E. Holt, Jr., and B. Barelare, Jr., *Proc. Amer. Physiol. Soc.*, April, 1937, pp. 132–133.

[5] L. R. Harris, Janet Clay, Florence J. Hargreaves and A. Ward, *Proc. Roy, Soc. London*, Series B, 113: 161–190, 1933.