gressive development of hardiness throughout the autumn period in the hardy varieties, while the tender varieties show no such change. At the end of November, the differences in exosmosis as measured by this method amount to several hundred per cent. between the very tender and the most hardy varieties. Colorimetric tests for chlorides and nitrates in the exudate from the frozen roots correlate very well with the conductivity measurements, and regeneration of growth in the greenhouse indicates that injury by freezing may be closely estimated by this method. The author plans to investigate its application to other plants and to perfect details of technique. The precise experimental method and results will be published soon.

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A SOURCE OF DIASTASE

EXPERIMENTS designed to illustrate digestion are not uncommon in beginning courses in botany, the action of diastase or amylase on starch paste being a common method of procedure. Ptyalin, contained in the saliva, may be successfully employed, but for classes involving large numbers of students this is difficult to obtain in sufficient quantity. Malt diastase may be prepared, if facilities are available, but the extraction must be performed with care, and the yield is small unless large-scale operations are employed. The ordinary commercial preparations, available on the market under various trade names, are unsuited, since they contain starch or sugar or both and thus vitiate the results before the experiments are started. After a considerable canvass of the situation, a source of diastase has been found which meets the requirements of a starch- and sugar-free enzym which is not only entirely suitable for experiments of the nature indicated, but which is useful in a number of physiological and biological procedures as well. Since it seems apparent that teachers generally are unaware of an easily obtainable enzym, this note may not be out of place.

The Digestive Ferments Company manufactures and sells a digestive ferment under the trade name of "Pangestin." The manufacturers claim that it is capable of converting eighty parts of potato starch into water soluble substances in five minutes in accordance with the U. S. P. X Revision test for pancreatin. Pangestin has good solubility, with a small amount of extraneous material not identified. Both the dry enzym and aqueous solution give a negative test for starch. Freshly made aqueous solution and the same after standing fifteen hours give a negative test for glucose. The digestive power has been tried on potato, corn and other starches in 2 per cent. aqueous paste form. Ten minutes at room temperature, and without an activator, give very positive tests for sugar with Fehling's solution. Cornstarch is apparently acted upon more rapidly than potato starch.

Pangestin is not a simple enzym. Not only is it strongly amylolytic, but, because it is of animal origin, it is also proteolytic, as shown by its power to digest completely the white of egg, and lipolytic, as shown by the hydrolysis of oils, such as cottonseed and olive. It is therefore a mixture of enzyms. This does not, however, detract from its usefulness, but rather enhances it. So far as the writer has been able to ascertain, Pangestin is the only trade product made in America conforming to the requirements of a starch- and sugar-free enzym, although several other chemical companies have signified their interest in the production of a purified product. One German product submitted has also been tested out, but has been found unsatisfactory.

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SPECIAL ARTICLES

NITROGEN FIXATION BY BLUE-GREEN ALGAE

NITROGEN fixation, a property common to several species of bacteria and a few fungi, has long been suspected as being a property of algae also. Considerable research by several investigators with green algae (*Chlorophyceae*) has failed to demonstrate that any one of these possesses the ability to use free nitrogen gas. The few claims that have been made to the contrary have been disproved, to the satisfaction of probably all the workers in this field. The writers have recently had occasion to check this point further. Three cultures of green algae, namely, *Chlamydomonas, Chlorella* and *Scenedesmus,* obtained from Cornell University through the courtesy of Dr. F. B. Wann, and a *Pleurococcus,* isolated from a local soil, were repeatedly tested for nitrogen-fixing powers, with negative results.

What are the facts regarding the nitrogen-fixing ability of the other common group of algae, known as the blue-greens (*Cyanophyceae*)? Many articles have appeared during the past forty years in which references are made to the fact that under various experimental conditions, particularly in greenhouse pots, there occurred gains in nitrogen or increased growth of higher plants where abundant growths of blue-green algae were present. In these greenhouse experiments the blue-green algae were growing in association not only with higher plants in most cases but with bacteria, fungi, green algae and all the other common soil micro-organisms. Hence it was impossible to attribute the fixation directly to the blue-greens. The fact that these forms were nearly always present in abundant numbers where the unexpected nitrogen fixations occurred naturally led many workers to suspect that they were the agents responsible. Others, however, stressed the fact that Azotobacter and other free-living bacteria were nearly always found closely associated with the dense algal growths. This observation formed a basis for the other commonly accepted idea that a sort of symbiotic relationship exists between the two types of organisms. In such a symbiosis the algae by means of their chlorophyll are supposed to furnish carbohydrates for the non-symbiotic bacteria, while the latter fix nitrogen, a portion of which the algae use. During the past few years this symbiotic theory has probably been more generally accepted than that of direct fixation by algae, because no one could demonstrate that any alga could use free nitrogen. In the case of blue-greens the failure to demonstrate fixation may be attributed largely to the difficulty of securing pure cultures. In the gelatinous algal sheaths, bacteria thrive and are eliminated only with great difficulty by ordinary bacteriological methods.

A few years ago the writers began nitrogen-fixation studies with blue-green algae, isolated from a local soil. In preliminary culture work these organisms in mixture with other soil organisms were found to fix as high as 40 mg nitrogen per 100 cc of nutrient solution, containing 1 per cent. sucrose, when exposed to sunlight in the laboratory window for approximately a year. These crude cultures were partially purified by being repeatedly plated out. In order to make the final separation, the algal cultures containing bacteria were exposed to ultra-violet light from a quartz mercury vapor lamp for periods varying from one to twenty minutes. Most of the bacteria were killed by exposures longer than five minutes, but several attempts had to be made before complete sterilization resulted without appreciable injury to the algae. In January, 1929, pure cultures were finally secured by use of this method. Freedom from bacteria and other organisms was determined by inoculation onto four commonly used bacteriological media, as well as by repeated direct microscopic examinations. When grown on a nitrogen-free medium through which a stream of air, purified by passing through sulphuric acid and sterile water, was constantly bubbled, the organisms were demonstrated to possess the ability to fix atmospheric nitrogen. During a period of 75 days' incubation, an average fixation of 5 mg nitrogen was obtained per 100 cc of a culture medium originally containing no nitrogen or sugar. Where one gram of sucrose was added the fixation was 8.5 mg nitrogen. The cultures were grown at room temperature in a southwest window where they received direct sunlight for approximately three hours each day. The growth requirements for the blue-green algae used are very simple; a complete nutrient medium usually produces the best growth, but even tap water, containing .05 per cent. K_2HPO_4, with a satisfactory light source produces a rather good growth. In the absence of light, if sugar is present in the medium, the organisms live for months and retain their normal color, but the growth is very slight. In the dark, fixation was so slight as to be almost within experimental error, but growth even in the presence of combined nitrogen was also slight. Additional experiments to determine this point are in progress.

The identification of the alga used in most of the experiments has not as yet been definitely made. Professor W. R. Taylor, of the University of Pennsylvania, is giving a very generous portion of his time in helping out on this phase of the work. He reports that because of the fact that it has grown for such a long time on artificial media, it is in many respects rather abnormal. Considerable additional culture work will be necessary to identify definitely the organism but, in general, Professor Taylor states that its characteristics agree rather closely with those of *Anabaena variabilis* Kütz. Another culture corresponds in most respects to *Phormidium molle* (Kütz) Gom. These names are given here merely as provisional designations until further studies are completed. The point of immediate importance is not the exact identification of the nitrogen-fixing species but rather the fact that a pure culture of a blue-green can use free nitrogen gas.

It is not the intention of the writers to leave the impression that this is the first time that blue-green algae have been grown in pure culture or demonstrated to use free nitrogen. Pringsheim[1] in 1912 was probably the first to grow blue-green algae, *Oscillatoria* and *Nostoc* species, free of bacteria. He did not demonstrate that either could fix nitrogen. A short time after the writers had obtained *A. variabilis* Kütz in pure culture and while a series of experiments was already under way to test its ability to use free nitro-

[1] E. G. Pringsheim, "Kulturversuche mit chlorophyllführenden Mikroorganismen. Zur Physiologie der Schizophyceen," *Beitr. Z. Biol. d. Pflanzen*, Bd. 12, 1913: 49–108.

gen, the recent excellent paper of K. Drewes[2] came to our attention. He grew *A. variabilis, Nostoc punctiforme* and another species of *Anabaena* in pure culture and obtained about 2 to 3 mg of nitrogen fixed per 250 cc of nitrogen-free nutrient solution during a period of two months. It is quite a coincidence that workers so far separated should have isolated what appears to be the same organism from different soils and independently demonstrated its powers of nitrogen fixation at so nearly the same time. The results agree very closely except that the quantities of nitrogen fixed were relatively larger in our results, 1.67 mg nitrogen per 100 cc per month in a sugar-free solution as contrasted with a corresponding figure of 0.6 mg nitrogen in Drewes' results. This point is not important, however, since the light intensity, quantity of CO_2 available, temperature and amount of inoculum are all important factors affecting the total fixation.

The economic significance of these findings remains to be determined, and work is being continued in this laboratory in the hope of learning more about the factors that influence the fixation, importance in nature and mechanism of the process. The fact, now definitely proved, that a chlorophyll-containing plant growing in pure culture can fix nitrogen in quantities far greater than experimental error puts additional emphasis upon the old question as to whether higher green plants in pure culture can use free nitrogen. Undoubtedly, more nitrogen is now being fixed in nature than can be accounted for by any previously known agencies of fixation. This statement is based largely upon the large energy requirements of non-symbiotic nitrogen-fixing organisms. The blue-green algae get most of their energy from sunlight, and the nitrogen that they fix costs little. We know that many species of these organisms are found in almost all soils and in fresh water, but their importance is largely unknown. It is, at least, significant that Robbins[3] in his study of the algae of Colorado soils, where nitrogenous salts often accumulate to such an extent as to cause "niter spots," isolated twenty-one species of algae, all but two of which were blue-greens. The most prevalent organisms were *Phormidium, Nostoc, Anabaena, Nodularia* and *Stigonema*. Robbins did not, of course, know that many or perhaps all of these forms are able to fix nitrogen. He considered that they are of importance in nitrogen fixation because they furnish energy for Azotobacter.

In the light of these results it is likely that blue-green algae are important non-symbiotic nitrogen-fixing organisms. They may, indeed, be the most important nitrogen-fixing agents in many agricultural soils.

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[2] K. Drewes, "Über die Assimilation des Luftstickstoffs durch Blaualgen," *Centbl. Bakt., Par.*, etc., 2. Abt., Bd. 76 (1928): 88–101.

[3] W. W. Robbins, "Algae in Some Colorado Soils," Colorado Agr. Exp. Sta. Bull. 184 (1912): 24–36.

SALINE DRINKING WATER

BECAUSE of the ease of modern modes of transportation, man of to-day is exposed to an ever-increasing change in living conditions to which he must adjust himself. Without a doubt, water is the greatest variable which one encounters in passing from one region to another, and it is this variable which is responsible for the greatest physiological changes in the body.

The waters of certain areas of the United States are either quite free from dissolved salt or contain only very small amounts. Such waters are called "soft" waters. In contrast to these practically salt-free waters are those waters which have come into contact with limestone beds and have taken up calcium carbonate in the form of the bicarbonate. Such waters are said to have temporary hardness. Surface waters which have passed through alkaline soils, or well and spring waters which have come into contact with salt beds, will dissolve these salts and will consequently have a brackish taste.

Large sections of the southwestern part of the United States have alkaline soils which impregnate surface waters with salts, and in addition there are in these regions extensive salt beds which contaminate the deep wells and many of the springs. The salt content of the rivers in these sections is often so high that it will kill vegetation along their shores. The salts present in these waters are sodium chloride, calcium chloride, calcium sulphate and magnesium sulphate. Analyses of waters from such sections have shown these salts in certain instances to be present in excess of 200,000 p.p.m., and 5,000 to 50,000 p.p.m. are common.

The question of the possible deleterious effects of such waters upon the animal organism immediately presents itself. Travelers drink such salty waters with considerable hesitation on account of their unpleasant taste and disagreeable physiologic action. Live-stock growers frequently attribute the death or poor condition of their stock to the salty waters which the animals must drink. This is especially true where the cattle drink from small streams which receive waste saline waters from the oil wells.

Strange as it may seem, scientific literature does not answer this vital question. There are volumes of semi-popular articles dealing with the medicinal properties of certain spring water. In the pharmaceutical litera-