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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October 9, 1929

[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 literature the physiological action of certain salts is recorded, but practically nothing can be found in regard to the physiological effects of the ingestion of saline drinking water. In a pamphlet published in 1925 by the U. S. Public Health Service, "Drinking Water Standards," an arbitrary standard is set for waters which are to be used on interstate commerce carriers, but it is a matter of common knowledge that much of the water being used continually by people in the southwest contains more saline matter than is permitted by the U. S. Health Service, and, nevertheless, there is no evidence of any deleterious effects arising as a result.

An investigation was begun in an attempt to answer the following questions in regard to saline waters. 1. What is the effect of the presence of considerable quantities of salt in our daily drinking water? 2. At what percentage does salt become deleterious to the organism? 3. Is it possible for the organism to adjust itself in time to saline drinking waters? The experimental animals used in this work were rats and other small animals. They were placed upon well-balanced rations supplemented with drinking water which was prepared by dissolving different amounts of various salts in distilled water. Sodium chloride was added to the water in amounts ranging from 500 p.p.m. to 50,000 p.p.m. Similar series were tried using calcium chloride, magnesium sulphate, sodium carbonate, sodium bicarbonate and calcium sulphate (up to the limits of saturation). The general appearance of the animals, their growth rate, their ability to reproduce and to rear their young and the condition of their organs at death were considered in studying the effects of the various salts upon the animal.

The first point determined was the maximum percentage of a single salt which could be administered in the water without producing harmful effects upon the animal. Mixtures of the salts were also added to the water to determine whether there might possibly be an antagonistic action between the various ions: that is, whether the deleterious effect of one ion might not be offset by the effect of a second ion. Such an antagonistic ion effect has been observed in certain body processes and also in plant nutrient solutions between sodium, calcium and magnesium ions.

From the data obtained, it is possible to postulate that 15,000 p.p.m. of magnesium sulphate has a retarding effect upon growth and that increases the mortality rate of the young. Twenty-five thousand p.p.m. of magnesium sulphate have a decided toxic effect. Calcium chloride produces even more harmful effects. No normal litters were found among rats drinking 10,000 p.p.m. of this salt; 15,000 p.p.m. lessen the growth rate, and 25,000 p.p.m. produce death. Ten thousand p.p.m. of sodium chloride affect reproduction unfavorably; 20,000 p.p.m. cause the growth of the animal to be stunted, and 25,000 p.p.m. produce a rapid decline in weight. Sodium carbonate is decidedly deleterious at a concentration of 20,000 p.p.m., and reproduction was interfered with at quite low levels. Sodium hydrogen carbonate is less injurious to the animals. A combination of 20,000 p.p.m. of sodium chloride and 5,000 p.p.m. of magnesium sulphate or calcium chloride inhibits growth. The results of feeding various other combinations of salts seem to indicate that in animals there is no antagonistic action between the ions such as there is in plants. The chlorine ion appears to be more toxic than either the carbonate or the sulphate ion. The calcium and magnesium ions are more harmful than the sodium ion. This can be explained by certain known physiological actions of ions. It is interesting to note that as the salt concentration increased the amount of water consumed by the animal daily also increased up to the point where the animal preferred to die of thirst rather than drink the necessary amount.

When animals which had been given a salty water were placed on distilled water soon after their first loss in weight, they rapidly regained their weight and appeared not to have been permanently injured by the saline content of the water. Animals which have received the salty water for some time seem better able to stand such water than do animals which are suddenly changed from distilled water to salty water.

The above results indicate that the continued use of saline solutions as drinking water produces injurious effects upon the animal organism. The concentration at which the solution becomes harmful varies with the particular salt used. The animal organism can apparently accustom itself within limits to a saline water, but in all probability it can not become adjusted to the effects of high concentrations.

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