in alcohol, but readily so in cold ether, and the evaporation of its ethereal solution yielded a yellow solid, resembling wax." Since Silliman's time practically no work has been done on the organic constituents in corals, which is rather surprising because the reef-building corals occur in almost unlimited abundance in the coastal regions of tropical waters.

In the course of a systematic study of the steroids of lower marine animals, the lipoid fractions of a number of stony- or reef-corals (Madreporaria) and sea-fans (Gorgonaceae) were prepared at this laboratory. It was found that these fractions represent a not inconsiderable proportion of the starting material. In the case of the staghorn coral, *Madrepora cervicornis,* concentration of its acetone extract led to the precipitation of a low-melting, crystalline, wax-like material in a yield of about 0.25 per cent. of the total mass of corals. After frequent recrystallizations the wax melted at 50–50.5°; it was identified as pure cetyl palmitate. The remaining extracts of the coral were saponified, and the non-saponifiable fraction, which amounted to 0.25 per cent. of the total, was separated into three fractions containing the following groups of compounds: (A) sterols precipitable with digitonine, (B) non-steroid alcohols, and (C) non-alcoholic compounds like hydrocarbons and ketones. Fraction A consisted of a mixture of sterols containing at least one unknown sterol giving an acetate of m.p. 176°. Fraction B consisted almost exclusively of cetyl alcohol and fraction C of low-melting hydrocarbons and small amounts of ketones. Similar results were obtained with the coral *Meandra areolata,* which contained about 0.3 per cent. non-saponifiable material consisting of cholesterol, an unknown sterol, cetyl alcohol, hydrocarbons and possibly some ketones. The results so far available indicate that stony corals contain from 0.3 to 0.5 per cent. of non-saponifiable material or about 10 per cent. of the total amount of organic material present.

The sea-fans (Gorgonaceae) which also contribute to reef formation are much more abundant in non-saponifiable material than the stony corals. In certain species the calcareous outside layers contain as much as 3 per cent. For example: 1,000 g. of dried and crushed external layers of a brown Gorgonia from Florida gave 33 g. of non-saponifiable material or 3.3 per cent. It consisted of about 20 per cent. of an unknown sterol, 50 per cent. of alcohols (cetyl alcohol) and 30 per cent. of semi-solid hydrocarbons. 1,100 g. of layers of a yellow Gorgonia gave 2 per cent. of non-saponifiable material consisting of about 30 per cent of an unknown sterol, 30 to 40 per cent. of other alcohols and the remainder of a mixture of hydrocarbons and possibly ketones which was liquid at room temperature. The compounds described can be isolated equally well from dried, but unbleached old museum specimens as from freshly collected material.

The presence of such sizable quantities of lipoid material in reef-building animals seems rather significant. It is not confined, as has already been pointed out by Silliman, to the relatively thin living layer, but extends deep into the entire structure of the coral. In view of these findings the idea at once suggests itself that coral reefs may act as gigantic accumulators of "wax-like" substances, especially of the chemically inert hydrocarbons. The bulk of the organic matter of dead marine animals and plants is probably brought back into circulation one way or the other. In the case of the reef-building animals, however, a significant portion of the organic matter becomes trapped in the ever-growing inorganic skeleton and hence is removed from further circulation. If this is indeed the case we must conceive the coral reefs as vast storehouses of compounds which may be considered as potential precursors of petroleum. At some time changes in the physical conditions may loosen this material from its inorganic surroundings and bring it to the surface, similar to the wax-like material which Silliman found floating on the surface of water in which corals had been boiled. The authors of the present article are not petroleum chemists and they do not claim the complicated problem of the formation of petroleum can be solved merely on the basis of the coral reefs. They present their preliminary findings only in order to call attention to the coral-reefs as one of the possible sources of petroleum and to stimulate discussion along this line.

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THE OCCURRENCE AND ISOLATION OF AZOTOBACTER IN CHINESE SOILS[1]

ON account of their relation to soil fertility, the non-symbiotic nitrogen fixation bacteria, Azotobacter, have received considerable attention since their discovery by Beijerinck in 1901. They are widely distributed in the soils throughout the world, as their occurrence has been reported in the soils of Java, India, Poland, the United States, England, Japan, Russia, etc. The only country whose soils are completely devoid of this microorganism is Finland.[2] As yet, however, there is no information about their occurrence in the vast area of Chinese soils.

The purpose of the present study is to make a general survey of the occurrence of Azotobacter in the soils of Szechuen Province, which, under the present circumstances, is probably the most important agricultural region in China. The work also involves the

[1] The detailed report of this work will appear in *Soil Bulletin* (bimonthly publication, National Geological Survey, Ministry of Economics, China).

[2] W. Brennar, *Geol. Komm. Finland Agrogeol. Meddel.*, 20: 1–15, 1924.

isolation of pure cultures from the soils for experimental works which the writer is planning to carry out in the near future.

The soil samples, including as many as fifteen or more types, were collected under more or less sterile conditions from various parts of the province, particularly the northwestern section. They are composite samples taken from the soils at a depth of about 20 to 30 cm below the surface.

The presence of Azotobacter in the soils was determined by using mannitol, dextrin or other simple carbohydrates in liquid media, and the results so obtained were checked by using either the nitrogen-free nutrient agar medium of Martin, Walker and Brown[3] or soil plaque methods of Winogradsky.[4] As a rule, two or three independent determinations were made for each sample.

Of all the 127 soil samples collected and determined, 102 or 78.2 per cent. contained Azotobacter. This finding is not surprising in view of the fact that in other countries over 50 per cent. of the soils containing Azotobacter is not uncommon, as reported by various workers, and furthermore, the soils of Szechuen Province are well known to be very fertile.

Isolation of the pure cultures was carried out during the progress of the survey. The media used were nitrogen-free agar media of various kinds. Repeated purifications were made by using congo red nitrogen-free agar medium of Bryan.[5] This medium proves to be very useful in purification especially in separating Azotobacter from their most common contaminants, Radiobacter.

The strains isolated from this survey are mostly *Azotobacter chroococcum* although a few of them are not yet definitely identified.

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PRODUCTION OF SYMPTOMS BY SUBCUTANEOUS INJECTION OF HISTAMINE WITHOUT INCREASE OF THE BLOOD HISTAMINE[1]

Histamine has been used to produce experimental shock by many investigators, and at one time it was thought to be one of the toxic factors responsible for the production of shock following trauma. Since it was impossible to detect histamine in the circulating blood under these conditions, this substance was rejected as a possible cause by many workers. It was stated that when symptoms of shock were produced by the injection of histamine, large amounts of this substance were present in the circulating blood and therefore easily detectable.[2]

In order to note the changes in the blood histamine following the production of symptoms by the subcutaneous injection of this substance, the following procedure has been carried out in ten patients. A control blood is taken, blood-pressure and pulse being noted. One mgm of histamine diphosphate is then injected subcutaneously and the blood histamine determined, at 5, 15, 30 minutes and one hour, blood-pressure and pulse being recorded at the same time. In each instance symptoms of histamine intoxication were noted, such as an increase in pulse, decrease in blood-pressure, flushing of the face and the onset of headache. In no case, however, was an increase of blood histamine observed, even at the height of the symptoms. It was also noted that the particular allergic symptoms were reproduced if the patient on whom the test was being carried out was an allergic individual. The relationship of blood histamine to allergy is being reported elsewhere.[3]

In the light of these findings, therefore, it appears that histamine intoxication may occur without an increase in the peripheral blood histamine. Failure to demonstrate an increase in the peripheral blood histamine does not exclude the possibility that this substance may be responsible for the symptoms of shock.

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AGASSIZ AND LIEBIG

The year 1940 should not be allowed to pass without noting that it marks the centenary of the publication of two papers whose contents have profoundly influenced the history of agronomy and of its offspring, soil science. These papers were "Etudes sur les glaciers," in two volumes, by the Swiss naturalist Louis Agassiz, and "Die Chemie in ihrer Anwendung auf Agriculture und Physiologie" by the German chemist Justus von Liebig.

Born within a few hundred miles of each other, the lives of these scientists were contemporaneous and almost conterminous. Agassiz, by his glacial hypothesis, solved the riddle of the origin of the surficial deposits of a large portion of the northern hemisphere. Liebig, with his mineral theory of plant nutrition, gave the world the scientific basis for the use of mineral fertilizers. Both men greatly stimulated research in their respective fields. Agassiz traveled in America, and liked the United States so well that he accepted the offer of a professorship in Harvard University and settled in this country.

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[3] Martin, Walker and Brown, *Research Bull.* 217, Iowa Agr. Exp. Sta., 1937.

[4] S. Winogradsky, *Ann. Inst. Pat.*, 40: 455–520, 1926.

[5] C. S. Bryan, *Soil Science*, 45: 1938.

[1] Aided by a grant from the Banting Research Foundation.

[2] C. A. Dragstedt and F. B. Mead, *Jour. Am. Med. Asn.*, 108: 95, 1937.

[3] *Journal Allergy* (in press).