direct assimilation or of digestion and assimilation. It includes the carbon compounds produced by photosyntax and many other substances, but is not applicable to CO_2 and H_2O, which are built into carbohydrates.

By these slight yet important changes in terminology we bring ourselves into harmony with the present knowledge of animal physiology, and have a much more intelligible and intelligent point of view from which to discern further truths regarding plant nutrition.

University of Wisconsin.

The bacterial flora of the Atlantic ocean in the vicinity of Woods Holl, Mass.

A contribution to the morphology and physiology of marine bacteria.

H. L. RUSSELL.

WITH PLATE XXXVI.

(Continued from p. 395.)

General biological features of the different organisms.

Zymogenic properties.

The majority of the bacteria isolated at Woods Holl belong to the liquefying group of micro-organisms and one of their fermentative actions is demonstrated in the production of a peptonizing enzyme that slowly liquefies gelatin. The digestion of the casein in milk cultures also attests the production of ferments that change the insoluble proteids into soluble peptones.

Pathogenic properties.

From the frequency with which bacteria are found in the water and mud of marine areas, it might be presumed that the organisms in question had no pathogenic properties but were purely saprophytic in their nutritive adaptation. This presumption however is not warranted on *a priori* grounds and it becomes necessary in working out the full life history of a micro-organism to test the relation of the germ as to its pathogenic properties. For this purpose, white rats were inoculated with one cc. of freshly grown bouillon cultures of the

different predominating forms but in no case was there any evidence that would lead one to think that any of the species tested possessed any pathogenic peculiarities.

Relation to gaseous environment.

Of the different forms isolated this season, almost all are decidedly aerobic. No growth takes place in plate cultures covered with sterilized mica sheets and the manner of growth in gelatin tubes demonstrates their predilection for oxygen. Only one species, B. litorosus, manifested any indifference to its gaseous surroundings. This species thrived quite as well along inoculation track as superficially, and in cultures prepared according to Buchner's pyrogallic acid method, a marked growth was to be noted in nutrient bouillon.

Effect on nitrate solutions.

The effect of bacteria upon the nitrogen-containing compounds is an important one. The fertility of soils depends in a large degree on the oxidizing action of certain bacterial forms. These convert the ammoniacal compounds into the more available nitrites and nitrates. Beside these oxidizing agents, however, there are a large number of germs that possess the antagonistic property of reducing these salts to simpler substances. The Franklands[10] and Jordan[11] have studied a number of forms that were isolated from the soil and the water supplies and noted their ability to reduce nitrate solutions to both nitrite and free ammonia.

The species now under discussion were also subjected to a similar line of experiment to see if they had any effect on the nitrates that might be in marine waters.

The nitrate solution used as a nutrient medium had the following composition: 1^{gm} pept. sicc. (Merck's); 1.01^{gm} KNO_3 (c.p.); 1000^{cc} Lake Michigan water.

This water was examined chemically and no trace of nitrogen in the form of nitrite was to be found. Erlenmeyer flasks containing one hundred cubic centimeters of this solution were used for cultures and after inoculating them with the various forms of micro-organisms they were incubated at 35°C. for a period of twenty-four hours and then analyzed for nitrites by the sulphanilic acid and naphthylamine hydrochlorate test.

[10] Zeits. f. Hyg. VI. 373.
[11] Rep't Mass. State Bd. of Health. 1890.

In each case a marked reaction was noted, showing that the nitrate solution had been reduced and that nitrites were present in varying amounts. After four days' incubation at 35°C., the cultures were analyzed quantitatively for U as nitrite by colorimetric standards with the following results:

Total am't of N as KNO in culture solution,	14.00	parts in	100,000.
N as KNO_2 in culture of Bacillus limicola,	1.26	" "	"
" " " " " " pelagicus,	2.8	" "	"
" " " " " " maritimus,	2.1	" "	"
" " " " " " litorosus,	2.3+	" "	"

This indicates that the amount of nitrogen converted into nitrite from nitrate as a result of four days' growth of bacteria is by no means inconsiderable; in one case, B. pelagicus, reaching one-fifth of amount available. The marked difference to be noted in the amount reduced by B. limicola and B. litorosus is not to be explained by the difference in the rapidity of growth but is due to relative differences in reducing powers. At 35°C., B. litorosus grows much more slowly than at the ordinary temperature of the room, while the reverse is true of B. limicola. This relative difference in rate of development is however in inverse ratio to the amount of nitrogen reduced and shows that the denitrifying ability of the two germs differs to a considerable degree.

In order to determine whether the reduction process is carried on until free N or NH_4OH is produced, another culture test was made. The production of NH_4OH from nitrate can not be recognized by nesslerization as it is impossible to distinguish between NH_4OH that might come from the organic N in the peptone and that from the nitrate or nitrite, but by using a known amount of nitrate as a culture medium and then determining the amount present at the end of experiment, one is able to ascertain whether any considerable percentage has been reduced.

A normal solution of $NaNO_2$ was made, to which one per cent. of dry peptone was added. This was used as a culture medium and cultures of the several forms were examined as to amount of nitrite present after they had incubated for a period of five days. Growth of germs was evident in cultures but in no case was there any diminution of the amount of nitrite present, so the conclusion was evident that while the several species here described were able to reduce nitrates to nitrites, the later stages of the reduction process of nitrate to gaseous N were not carried on by these forms.

Experiments on insolation.

Although the fact that direct sunlight has a deleterious effect upon bacterial life in general has been known for a number of years[12] the full significance of this has not been generally appreciated until very recently. Carefully conducted experiments have shown that many of the pathogenic forms are profoundly affected by direct insolation not only when they are in a vegetating but in a quiescent spore stage as well.

The marine species isolated at Woods Holl have been tested in order to see whether forms naturally saprophytes were as easily affected as those accustomed to a parasitic mode of life.

Bouillon cultures not more than twenty-four hours old were used as seed. From these control gelatin plates were made by using one loopful of diluted culture. Two parallel sets of bouillon tubes, each containing two or three cc. of fluid, were also prepared by using one loopful of culture as seed. One of these was then wrapped in several folds of black tissue paper, care being taken to insert small pellets of cotton batting between the black paper and the glass of the tube, so as to diminish the effect of heat absorption and conduction to culture liquid as far as possible. These parallel sets of cultures were then exposed to the effect of direct sunlight during the day. At the close of the experiment, the culture tubes were filled with sterile gelatin and plate cultures made, so that the total number of germs could be directly ascertained, and compared with the control plate that showed the approximate number originally seeded.

Results of experiment were as follows:

Series inoculated and exposed to direct sunlight.
April 10th, 9 A. M. Sky clear, temperature varied from 15–21° C.
April 11th, sky cloudy all day.
April 12th, sky clear all day, temp. from 21–25° C.
April 13th, sky clear (cloudy from 1–3 P. M.), temp. 20–23° C.

Series planted on April 14th. Bouillon in "darkened" cultures appeared turbid with bacterial growth when unwrapped; the "exposed" tubes were perfectly clear and apparently unchanged. These tubes were now filled with sterile gelatin and plate cultures made with the following results:

NAME OF GERM.	NO. OF GERMS PLANTED AS "SEED."	NO. OF GERMS IN DARKENED CULTURE.	NO. OF GERMS IN EXPOSED CULTURE.
Bacillus maritimus	15	Several thousand	0
Bacillus litorosus	25	Several thousand	1
Bacillus pelagicus	420	Innumerable	0

[12] Downes & Blunt, Proc. Roy. Soc. 1877.

This confirms the work of other observers and shows that marine organisms are likewise susceptible in a high degree to the direct rays of solar light and that in a vegetative condition, they are easily destroyed in this way.

Distribution of species.

The distribution of species may be considered in two ways: first, as to area covered or superficial distribution; second, as to depth or bathymetrical range. The region examined at Woods Holl covered a larger area than at Naples, but the opportunity for determining the bathymetrical range was unfavorable owing to the extreme shallowness of the sea at this place.

The observations this season cover rather more than 100 square miles of the region of Buzzard's Bay and Vineyard Sound. The water forms were found to be distributed throughout the water masses very generally, as was likewise the case with those species indigenous to the mud.

Through the kindness of Prof. Wm. Libbey, Jr., opportunity was offered for the collection of material from the sea bottom that was secured by him on board of the U. S. Fish Commission schooner *Grampus*, at a distance of one hundred miles from mainland (New Bedford). The mud, consisting of globigerina ooze, was taken at the depth of 450^{ft} on the edge of the great continental platform that skirts the eastern coast of the United States. Only two samples were collected, but these sufficed to show that two of the more common mud forms that were to be found nearer the shore were also inhabitants of this locality. This instance, although isolated, suffices to show that the forms found in the mud are not locally distributed, but are spread over a considerable extent of the sea bottom.

This point is more strongly brought out when we compare the bacterial flora of this side of the Atlantic with that of the Mediterranean. Bacillus limosus was one of the most prevalent of the mud forms that was isolated in Mediterranean waters, and this same species has been identified by comparison of pure cultures brought from Naples with a form that is a common inhabitant of the sea slime at Woods Holl. The probable range of this cosmopolitan species can only be conjectured. It is a common form at Naples at the depth of 3,500^{ft}, and was also found abundantly on this coast as well as in the samples taken on the *Grampus* at the distance of 100 miles from land. Its presence at these three isolated localities testifies that its distribution is very general in the Atlan-

tic at least. Aside from this species, another mud form was secured on the *Grampus* that bore a very close resemblance to B. granulosus, a prevalent Neapolitan species. Careful comparison on same culture media of the germs from the two sources showed only slight differences, but these were scarcely more than would be expected of specimens coming from widely separated localities.

Concerning the distribution of those species that are common to this locality but unknown in the Mediterranean bottom, some observations are to be noted. First there is a marked difference as to the habitat of different species. Some are found solely in the mud layers, others are common to both water and mud.

Bacillus limicola, a common chromogenic species inhabits exclusively the ground layers of the sea bottom. In the vicinity of Woods Holl, it is a common form and was found in shallow places adjacent to the coast as well as in the deeper mud layers at a distance of ten to fifteen miles from land.

Three other forms, B. pelagicus, B. maritimus and B. litorosus which comprise, with the above mentioned species, the major portion of the bacterial flora of this locality, were found in both the water and the underlying ground layers. These were distributed throughout the water at all depths and B. pelagicus and B. maritimus were abundant in the samples of mud taken on the *Grampus* at a distance of 100 miles from land and in 450 feet of water.

Karlinski[13] has put forth a theory based upon the examination of one of the fresh water lakes in Bosnia that there is a horizontal zonary distribution of bacterial forms in water masses. He found, even within a vertical range of seventeen meters, a marked separation of the bacteria according to species. Forms common at the surface disappeared entirely in the deeper layers and were replaced by species peculiar to submerged strata. These results contravene those obtained at both this locality and at Naples. Here the study of the bathymetrical range of the different species has been limited on account of the shallowness of the sea, but the opportunity afforded by the dredgings from the *Grampus* showed that two forms, B. pelagicus and B. maritimus, were present in abundance at the depth of 450 feet as well as in the mud banks on the shore.

Much more favorable conditions for studying this question

[13] Loc. cit.

were had at Naples as the depth of the sea at this point is not inconsiderable.

Three of the most prevalent forms found in the Mediterranean sea bottom showed a vertical range exceeding 3,500ft, for they had not disappeared from cultures made from mud taken at this depth, and it is probable that their bathymetrical range was even considerably greater than this.

Concerning the relation between the superficial strata and extreme abysmal depths, we know nothing. If the relation between the higher forms of animal life hold in reference to bacterial forms, the presumption would be that there is a marked difference as to species between these layers. But such an analogy is inadmissible unless based upon observations, and these as yet are lacking.

University of Wisconsin.

(*To be concluded.*)

Plants hurt by a late freeze.

P. H. ROLFS.

In observing the effects of a frost in late spring many strange freaks are noticed. On the night of March 4, 1893, the thermometer went down to 29° F. at Lake City, Florida. The opinions as to whether harm was done were as various as the locations heard from. Closer observations showed that the following plants had suffered:

The variety of *Brassica oleracea*, known as "collards," is extensively grown for the kitchen and market. With considerable surprise I found that the young plants which had been set out had their larger leaves frozen. Further search showed the older plants that had shot up to flower drooping over. These racemes did not recover.

The largest and most beautiful of our wild violets, *V. sagittata* Ait., had the expanded portion of the petals, and in some cases the stigma frozen. Where, however, there was a slight protection, as a clump of saw palmetto or a blue berry bush, no harm was done.

Of the ornamental trees, the China tree, *Melia Azederach* L., suffered the most severely. It had made a vigorous growth; some young shoots were five or six inches long. All that were on low limbs less than eight feet from the ground were cut back to last year's wood, while those that were twelve feet or more from the ground were left unharmed.

Of the genus *Citrus* both *C. aurantium* L. and *C. vulgaris*

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