blood picture seems to be significant in the dilute brown strain of mice. The general appearance of the animals is different from that observed in the rare cases that have occurred spontaneously in this strain.[6] The incidence, to date, is more than 20 per cent. and the time elapsed in the development of the lymphomatosis is one third of that required for the spontaneous disease to appear. Further studies are being undertaken to evaluate the many factors involved, and other strains of mice are being investigated in the same manner.

JOHN J. MORTON
G. BURROUGHS MIDER

## A PARADOX IN THE ADAPTATION OF MARINE BACTERIA TO HYPOTONIC SOLUTIONS[1]

ACCORDING to Korinek[2] marine bacteria are distinguishable from freshwater or terrestrial species on a basis of their salt tolerance. This is in agreement with the observations of ZoBell and Feltham,[3] who found that less than 10 per cent. of the bacteria isolated from the sea at places remote from terrigenous contamination can multiply in nutrient freshwater media, and a smaller percentage of bacteria from freshwater sources multiply in undiluted sea-water media.

In studying the factors which influence the specific salt requirements of bacteria from heterologous environments, attempts were made to acclimatize marine bacteria to hypotonic solutions by gradually diluting the sea-water medium with each successive transfer of the cultures. Twelve recently isolated species were selected which would grow in nutrient sea-water broth but not in similar freshwater media. The media consisted of 0.2 per cent. each of Bacto-peptone, proteose-peptone and beef-extract and 0.025 per cent. of ferric chloride in either sea water, distilled water or various dilutions of sea water. The pH, incubation temperature (22° C.) and other experimental conditions were the same with the different kinds of media. At the beginning of the experiments all twelve cultures multiplied in 75 per cent. sea-water medium (75 parts of sea water diluted with 25 parts of distilled water). Seven of them grew in 50 per cent. sea-water medium, but none of them grew in 25 per cent. sea-water medium.

As soon as the turbidity of the 75 per cent. sea-water broth indicated good growth, a loopful of a dilute suspension of each culture was transplanted to 70 per cent. sea-water broth. If after 24 to 48 hours there was evidence of multiplication, this in turn was used to inoculate 65 per cent. sea-water broth, and so on seriatim, decreasing the concentration of sea water each time by 5 per cent. Under these conditions all twelve cultures were readily adapted to 45 per cent. sea water. Upon the next transfer, however, three of the cultures failed to show any signs of growth in 40 per cent. sea water, even after a week's incubation, although in higher concentrations of sea water they rendered the media turbid with growth in 24 to 48 hours. Therefore transplants of these fastidious cultures were made from 45 per cent. to 42½ per cent. sea-water medium, and in each successive medium the concentration of sea water was decreased by only 2½ per cent.

Most of the cultures could be acclimatized to 25–30 per cent. sea-water media, but below this concentration considerable difficulty and delay were encountered in making them grow, although the concentration of the sea water was changed by decrements of only 2½ per cent. After five months of almost daily attention four of the original twelve cultures were multiplying in freshwater medium containing no sea water. The lowest concentration in which the others grew may be summarized as follows:

| | | | | | | |
|---|---|---|---|---|---|---|
| 2 | cultures | in | 5 | per | cent. | sea water |
| 3 | " | " | 10 | " | " | " |
| 1 | culture | in | 17½ | " | " | " |
| 1 | " | " | 22½ | " | " | " |
| 1 | " | " | 25 | " | " | " |

In each case attempts to induce growth in the next lower dilution were negative, and the growth in the above media was very meager after 48 hours.

At this stage the parent stock cultures, which had been maintained in the refrigerator on sea-water agar during the five-month period, were reexamined. Microscopic inspection of stained smears revealed that the acclimatized cultures did not differ morphologically from their parent cultures. Paradoxically, though, it was found that all except three of the parent cultures multiplied when transplanted to freshwater medium and these three multiplied in 10 per cent. sea-water medium. Similar results were obtained with the original sea-water broth cultures, which had been maintained at room temperature since the beginning of the experiment, although the salt content of the medium had actually increased due to the evaporation of water. In other words, the old stock cultures were more euryhaline and better adapted to grow in hypotonic solutions than their progeny, which had been rejuvenated almost daily in attempting to gradually acclimatize them to lower salinities.

A repetition of the experiment with other recently isolated marine bacteria yielded similar results. In fact, tests on over a hundred different pure cultures of bacteria, which upon initial isolation from the sea

[1] Contributions from the Scripps Institution of Oceanography. New Series, No. 16.
[2] J. Korinek, *Centralbl. f. Bakt.*, Abt II, 71: 73, 1927.
[3] C. E. ZoBell and C. B. Feltham, *Proc. Fifth Pacific Sci. Cong.*, 3: 2097, 1933.

grew in sea-water medium but not in freshwater medium, revealed that after two to five years' maintenance in sea-water media virtually all of them would grow in freshwater medium.

At first this seems contrary to the observations of Kluyver and Baars[4] that bacteria become less adaptable to adverse environmental conditions during prolonged laboratory cultivation. Moreover, it is contrary to the accepted beliefs concerning the adaptability of organisms in general. However, there are several factors which must be taken into account in the interpretation of this apparent paradox. According to Rahn[5] it is established that physiologically young bacteria are more sensitive to adverse conditions than old ones. The frequent transfer of the cultures during the attempts to acclimatize them to hypotonic solutions tends to keep them physiologically young, while the stock cultures becomes senescent.

Data on the salt tolerance of bacteria from different strata of mud cores collected from the deep sea bottom lend credence to this "tolerance with senescence" theory. The majority of the bacteria found in the topmost few millimeters of sedimentary material where conditions favor their multiplication are typical marine species which grow in sea-water but not in freshwater media upon initial isolation.[6] However, in the deeper strata where the aerobic bacteria have probably been buried in a state of suspended animation for a long time lacking both oxygen and food for their multiplication, the majority of them grow equally well on sea-water and freshwater media. Similarly, the senescent bacteria from these deeper mud strata will grow over a wider temperature range than the physiologically young psychrophilic bacteria found in the topmost layers of mud.

It has been suggested that these bacteria require for growth certain allelocatalysts or accessory growth substances, which are present in sea water but not in fresh water. In an old culture, these substances may accumulate to such an extent that an inoculum therefrom may carry into a new culture an amount sufficient to promote growth, even though the new medium fails to contain the necessary substances. This, perhaps, is the reason for the greater tolerance of these senescent cultures to fresh water. This possibility, as well as others, is being investigated in order to account for the phenomena reported above.

CLAUDE E. ZOBELL
H. DAVID MICHENER
SCRIPPS INSTITUTION OF OCEANOGRAPHY,
UNIVERSITY OF CALIFORNIA, LA JOLLA

[4] A. J. Kluyver and J. K. Baars, *Proc. Koninklijke van Wetenschappen te Amsterdam*, 35: 1, 1932.

[5] O. Rahn, "Physiology of Bacteria," P. Blakiston's Son, Philadelphia, 1932.

[6] C. E. ZoBell and D. Q. Anderson, *Bull. Amer. Assoc. Petrol. Geol.*, 20: 258, 1936.

## THE PROTEIN CONTENT OF MOSAIC TOBACCO[1]

THE concentration of virus protein in tobacco infected with mosaic and the proportion of virus protein to normal proteins are data necessary to an adequate understanding of the synthesis of virus protein. According to Bawden and Pirie,[2] mosaic plants may contain five to ten times as much protein as normal tobacco, and according to Stanley,[3, 4] as much as 80 per cent. of the total protein of diseased tissue may be virus protein. Either an enormous accumulation of virus protein or an almost complete change of normal into virus protein has to be assumed. Such estimates are based, however, on the yield of protein obtained by extraction, and, especially with healthy plants, the extractable protein accounts for only a small fraction of the total nitrogen. On the other hand, Stanley is less concerned with the comparison of healthy and diseased tissues than with the examination of the diseased tissues as such. However, in spite of an increase in extractable protein in virus-bearing plants, a comparison of diseased and control plants made from the recent data of Stanley[3] appear to show no significant increase in total nitrogen due to the disease. With some strains of mosaic a decrease has occurred. This follows from the published data when the residual nitrogen in the press cakes is taken into account. For instance, in plants infected with common mosaic, the total nitrogen was 7.25 mg/g, while control plants contained 6.90 mg/g. Plants infected with aucuba mosaic contained 2.92 mg/g, as compared with the same control, thus indicating a substantial decrease.

The extraction of the protein, on the other hand, may have been incomplete. The quantities obtained from healthy plants are barely a tenth of the protein content of tobacco reported by Vickery and Pucher[5] from an extensive series of analyses, also on healthy plants. The highest yield recorded by Stanley shows 22 per cent. of the total nitrogen as protein in diseased plants, while the lowest yield was only 5 per cent. in control plants.

By applying the observation[2, 6] that virus protein is not attacked by crude trypsin, although normal tobacco proteins are hydrolyzed, it has been possible to develop a method of distinguishing between trypsin-resistant virus protein and normal proteins without resorting to extraction of the tissue.

[1] Food Research Division Contribution No. 362.

[2] F. C. Bawden and N. W. Pirie, *Proc. Roy. Soc.*, B 123: 274, 1937.

[3] W. M. Stanley, *Phytopath.*, 27: 1152, 1937. (Particularly page 1152 together with page 1155.)

[4] W. M. Stanley, *Jour. Biol. Chem.*, 121: 205, 1937.

[5] H. B. Vickery and G. W. Pucher, *Conn. Agr. Exp. Sta. Bull.* No. 324, 1931.

[6] L. F. Martin, H. H. McKinney and L. W. Boyle, SCIENCE, 86: 2234, 380–381, October 22, 1937.