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Observations on the Nutrition of *Flabellula mira* Schaeffer and other Amoebae.

By

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The work of various investigators (Beyerinck, 1896; Tsujitani, 1898; Mouton, 1902; Oehler, 1916; Castellani, 1930; and Rice, 1935) has shown that a number of species of amoebae were capable of utilizing pure strains of bacteria as food, and that such a diet was adequate if continued throughout many generations. However, Frosch (1897) found that *Amoeba nitrophila* grew better on one kind of bacterium than any other, and Oehler (1916) reported that his cultures of amoebae grew better on gram-negative bacteria than on gram-positive organisms. *Flabellula mira*, a marine amoeba, gave excellent cultures when a diet of pure strains of *Pseudomonas striata*, *Flavobacterium denitrificans*, and *Achromobacter* sp. was used (Rice, 1935).

In order to discover whether *Flabellula mira* and three other marine amoebae could utilize pure strains of a number of species of bacteria, the present work was undertaken.

Stock cultures of *Flabellula mira* were obtained from Dr. D. L. Hopkins. By methods described in the preceding paper, pure cultures of *Rugipes vivax* Schaeffer, a large limax amoeba, and a small limax amoeba were obtained. All of these amoebae are marine forms. They were cultured on 1.5 per cent agar plates containing 0.05 per cent dextrose and 0.025 per cent peptone in artifical sea water (Mc Clendon, Gault, and Mulholland, 1917).

Stock cultures of *Sarcina subflava*, *Aerobacter aerogenes*, *Bacillus subtilis*, *Escherichia coli*, *Serratia marcesens*, *Serratia ruber*, and *Staphylococcus albus* were obtained from Dr. F. A. Wolf. Stock cultures of

Eberthella typhi and *Eberthella dysenteriae* were furnished by the Department of Bacteriology of the Duke University School of Medicine. An unidentified bacillus, which produced brown pigment, was also obtained in pure culture. All of the bacteria were transferred to agar slants of medium made up as described above. Although they were somewhat depressed at first by the salinity of the medium, good cultures gradually developed.

Manipulations of cultures were carried out with the strictest precautions in order to avoid contamination. All inoculations were made with sterile platinum loops. Samples from monobacterial cultures were plated from time to time to establish their purity. Glassware was thoroughly washed with soap and water, immersed in sulfuric acid-potassium dichromate cleaning solution for a few minutes, and then rinsed in five changes of distilled water.

Forty sterile agar plates were prepared from the medium already described. Ten plates were inoculated with *Flabellula mira*, ten with *Rugipes vivax*, ten with the large limax amoeba, and ten with the small limax amoeba. The inoculations were made near one side of the plates. One plate in each set of ten was inoculated then with *Sarcina subflava* by making a series of parallel streaks from a point near the amoeba inoculation to the opposite side of the plate. Similarly one plate in the set was inoculated with each of the remaining bacteria. Table 1 shows how inoculations were made for both bacteria and amoebae. The plates were then examined bottom side up under the microscope to determine the aproximate number of amoebae present.

After 24 hours the plates were examined again under the low power of the microscope. In every case the amoebae had begun to multiply at the original point of inoculation, and migrate radially on the agar surface. After 48 hours large numbers of amoebae were present at this point, and migration had progressed so that some amoebae had reached the first streak of pure strain bacteria, and, in a few cases, the second streak. In some cases the amoebae had begun to multiply in the streaks, in others not. These latter were characterized by emaciation, in many instances not being more than one half the size of the original ancestors at the inoculation point. The results of this experiment are given in Table 1, which is based on three repetitions of the same experiment. Variation in the ability of the amoebae to utilize the same and different organisms is apparent. In no case, however, was *Serratia ruber* satisfactory as a food organism. *Flabellula mira* and *Rugipes vivax*, both

Table 1.

Bacterium	Amoeba			
	F. mira	*R. vivax*	*L. limax*	*S. limax*
* *Sarcina subflava*	A	B	—	—
Aerobacter aerogenes	A	A	A	A
* *Bacillus subtilis*	—	B	B	A
Escherichia coli	A	A	A	B
Serratia marcesens	A	A	A	A
Serratia ruber	—	—	—	—
Unidentified bacillus	—	B	B	A
* *Staphylococcus albus*	B	B	A	A
Eberthella typhi	A	A	—	A
Eberthella dysenteriae	A	B	—	B

Explanation of Table.
A Excellent growth, B Good growth, — No growth, * Gram positive.

of which are fan-shaped amoebae and locomote by means of a single wave-like pseudopodium, varied somewhat as compared with each other and with the limax amoebae. *Aerobacter aerogenes*, *Escherichia coli*, and *Serratia marcesens* seemed to be satisfactory as food in every case. Whether bacteria were gram-positive or gram-negative seemed to make little difference in the ability of the amoebae to utilize them as food. Pathogenic organisms appeared to serve for food as well as free-living forms.

After one week the amoebae in all successful cultures had migrated to the last streak. In the course of migration each streak showed successively an increase in numbers of amoebae until these almost covered the streak as a layer of contiguous cells, followed by a gradual decrease in size of the amoebae, and finally by encystment. Those amoebae which had migrated in such a way that they had missed the streaks decreased in size and encysted. This was also true of the amoebae which were unable to utilize certain strains of bacteria, e. g. the large limax amoeba when migrating through *Sarcina subflava*. Such cultures were discarded. That failure of the cultures was due to the inadequacy of the diet was shown by the fact that amoebae multiplied rapidly on the contaminants at the original point of inoculation, but, having reached the streaks of pure strain bacteria, gradually became emaciated and encysted. Three repetitions of the experiments yielded the same results.

The amoebae from the last streak in successful cultures were found, by plating samples, to be living on pure strains of bacteria. These amoebae and their bacterial food were used as inocula for

subcultures, which were in turn subcultured for a period of a month. Examination showed numerous amoebae which were growing and reproducing with undiminished vigor.

The subcultures were set up in the same way as the original cultures, so that amoebae migrated across a series of parallel streaks of the desired bacterium. The migration results in the amoebae losing their contaminating bacteria, the streaks of bacteria of pure strain inhibit the growth of contaminants. The continuance of this method of subculture, even after monobacterial cultures of amoebae have been developed, aids in the loss of any stray contaminant which may not have been shown by plating samples.

In summary, it may be said that the present work is in agresment with that of the majority of workers in showing that the diet of most amoebae is rather extensive. *Flabellula mira* (Rice, 1935) had been shown to utilize three different pure strains of bacteria. The list has now been extended to thirteen pure strains. Three other marine amoebae have also been grown on a number of pure strains. Oehler (1916) found that his cultures of amoebae thrived better on gram-negative than gram-positive bacteria. *Flabellula mira*, *Rugipes vivax*, a small limax amoeba, and a large limax amoeba utilized both equally well. Three pathogenic bacteria also served as an adequate diet. Only one bacterium, *Serratia ruber*, was found to be unsatisfactory as food in every case.

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