on fine linen, dried at 37° for two days, ground in a mortar until it would pass a 60 mesh sieve, and divided into four portions. The first was dried at 100° to constant weight which was 45.7 per cent. of its original weight. The second, without preliminary treatment was extracted in a soxhlet with ether then with alcohol, and again with ether. The ether extract of the alcohol extract was added to the other ether extracts and dried. The result was 7.48 per cent. of the original dry weight of the powdered mycellium. The third portion treated by the alcoholic ammonia method, gave 8.45 per cent.; the fourth portion treated by the HCl method gave 10.13 per cent. An aliquot part of this last sample, which was not dried, but was saponified with alcoholic potash yielded 38.7 per cent unsaponifiable matter.

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Some observations on pellicle formation.

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Bacillus subtilis characteristically produces a diffuse turbidity on broth, which usually clears toward the end of the first twelve hours, the organisms floating on the surface in small islands. These later grow together producing a heavy wrinkled pellicle. It seemed therefore that a study of possible factors influencing this spontaneous migration to the surface might illuminate the subject of pellicle-formation and surface growth in general.

Equal amounts of a young diffuse culture of B. subtilis were introduced into tubes containing each 10 c.c. of ordinary broth. These were incubated and at hourly intervals for 36 hours observations were made of morphology, progress of growth, spore formation, buoyancy of the pellicle and surface tension of the medium. The surface patches appeared at 10 hours, and the pellicles were well formed at 15. The first positive heat test for spores was obtained at 20 hours, but the cultures were not con-

sistently positive until after the 24th observation. At the 25th hour the first spores were observed in stained preparations. At all times the majority of the growth could be centrifuged to the bottom of the tube, showing that the organisms are heavier than the medium. Occasionally small rumpled fragments of the pellicle remained at the top of the centrifuge tube, but these may well have imprisoned air bubbles.

Larson[1] has shown that this organism does not form pellicles on media whose surface tension has been sufficiently reduced by soap. The question naturally arises whether the diffusely growing organisms exhaust some surface tension depressant in the media prior to their upward migration. This, however, is not the case, since the surface tension remained approximately 59 dynes throughout the period of hourly observation (36 hours) and was still the same 13 days after inoculation, at which time the pellicles sank spontaneously.

The avidity which this organism is supposed to have for oxygen, is apparently not a factor, since cultures in large flasks with oxygen bubbled up continuously from the bottom still exhibit surface growth.

A significant observation is that the upper surface of young pellicles is not wetted by water, although ethyl ether, petroleum ether, and chloroform spread upon it readily. It is impossible to invert a floating pellicle. If one lifts it and attempts to replace it upside down on the water it at once rights itself and continues to do so, until repeated manipulation has injured and wetted the surface originally uppermost. In such a case the pellicle usually slowly sinks. Experiments are now under way to determine whether this behavior is due to an accumulation of old cells storing lipoids in the upper layers of the pellicle.

[1] *Jour. Inf. Dis.*, 1919, xxv, 1.