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Studies on quantitative determination of fat in micro-organisms.

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Great difficulty is experienced in extracting fat from wet or dried micro-organisms. It has been suggested that a large portion of the fat they contain may be held in physical or chemical combination by some ingredient of the protoplasm and various preliminary treatments have been developed to free it from such combination. Three of the simplest are that of Larson and Larson for bacteria[1] which depends on simultaneous drying and extraction by acetone, followed by ether extraction of the solid residue and the acetone extract; I. S. MacLean's method used on yeast[2] which consists in boiling with normal HCl, washing and then extracting with ether in a Soxhlet; and the method developed by C. R. Smith for work on edible pastes, in which he boils the sample with alcoholic ammonia, and then extracts with ether.

The work here reported was done on Oidium Lactis. In the first experiment a two weeks' growth was drained by suction and divided into three portions, one of which was treated by each of the above methods without previous drying. The residues as well as the extracts were dried to constant weight and the total dry weight of the samples obtained by addition. The MacLean method is impractical on moist samples, as an unmanageable mucilaginous brown material results from the acid treatment. The acetone method gave 1.21 per cent. ether extract; the alcoholic ammonia method gave 6.13 per cent. No further studies were made on the lipoids thus extracted, as drying to constant weight, either in an oven at 100° or in a vacuum dessicator over P_2O_5 at room temperature, results in a hard, semitransparent brown material, insoluble in petroleum ether, only a portion of which is soluble in ethyl ether.

In another experiment, a three weeks old growth was spread

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1 *Jour. Inf. Dis.*, 1922, xxxi, 407.

2 *Biochem. Jour.*, 1922, xvi, 370.

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-