by producing the temporary appearance of large lipid particles in the blood, causes the normal defense mechanisms of the intima to retain some of these particles and thus gradually and infinitesimally to build up the full picture of stenotic and occlusive arterial disease.

The pathogenic mechanism and etiology of atherosclerosis will be considered in greater detail in other communications from this laboratory.

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The Production of Mushroom Mycelium (*Agaricus campestris*) in Submerged Culture

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Commercial mushroom production in the United States is accomplished exclusively by growing the mushroom, *Agaricus campestris*, on composts, usually prepared from horse manure. The purpose of this paper is to present the first instance in which the mycelium of this mushroom has been grown on liquid medium under submerged fermentation.

Since the mycelium produced by this technique was obtained in good yield and was found to have the characteristic mushroom flavor, it seems possible that production in submerged culture will have considerable application in the manufacture of mushroom soups, gravies, and flavorings and in the production of spawn for seeding commercial mushroom beds.

For the preliminary tests, a strain of *A. campestris* was isolated from a white mushroom of this species taken from a commercial mushroom bed. A good growth of mycelium was obtained on wort agar. The mycelial mat was broken up in a sterile Waring blendor, and a sterile culture medium was inoculated with the suspension. The inoculated medium was then transferred to the fermentor and incubated at 25° C until a heavy growth of finely divided hyphae had been obtained. For tests on a small scale the fermentor equipped for rapid agitation during aeration and described by Feustel and Humfeld (*1*) was used, while for production in larger amounts the fermentor designed to draw in air by suction during agitation as described by Humfeld (*2*) was employed.

Good growth of mushroom mycelium was obtained on media containing either asparagus butt juice or press juice from pear waste as the main substrate. The addition of inorganic salts was essential in the pear juice medium. It was also found that fair yields could be obtained in media consisting of monosodium glutamate, dextrose, and inorganic salts.

After the culture was harvested, the mycelium was separated from the culture liquor by centrifuging. The mycelium was resuspended in water and recentrifuged. The samples made up in the preliminary work were dried from the frozen state.

Yields of mycelium up to 60% by weight of the sugar consumed have been obtained. The growth from a comparatively small inoculum is rather slow as compared to that of yeast; however, a continuous-fermentation experiment indicated that, once the maximum cell volume consistent with the composition of the medium has been attained, from one-half to three-quarters of the culture medium may be harvested and replaced with fresh sterile medium every 6–12 hrs of operation. Successful operation by this method has been accomplished over a period of 6 consecutive days. For instance, in one experiment, 3.75 liters of culture medium, which contained 20 gm of mycelium on the dry basis, was built up in 33 hrs to a volume of 16 liters with a dry weight of mycelium of 421 gm. At this stage 8 liters of the culture was harvested and 8 liters of fresh medium added to the 8 liters remaining in the fermentor. Maximum cell volume was attained in 12 hrs. This procedure was repeated, and there was no indication that such operation could not be continued indefinitely. This and other experiments indicate that no serious difficulties should be encountered in adapting the process to economic commercial production of mushroom mycelium.

The chemical compositions of commercial mushrooms and the mushroom mycelium produced by submerged fermentation were found to be very similar. McConnell and Esselen (*3*) report that *A. campestris* mushroom contains protein (N × 6.25), 35.6%; fat (ether-soluble material), 2.3%; and ash, 10.2%, calculated to a moisture-free basis. The mushroom mycelium produced by submerged fermentation, calculated to the same basis, contained protein, 49.1%; fat, 3.1%; and ash, 8.1%. It may be noted that the mycelium was somewhat higher in protein and fat content, and lower in ash. Other constituents such as carbohydrates and fiber have not been determined.

The adaptation of the submerged-culture process to the production of mycelium of the higher fungi would seem to offer the possibility of industrial-scale application to an extensive group of organisms. Such application may include the production of enzymes such as cellulase, solvents, antibiotic agents, and substances of pharmaceutical significance. Each species would probably need considerable investigation to determine optimal conditions for its propagation and for the production of such desired substances.

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