

in certain outbreaks is attributable not only to the microorganism, but also to certain of the materials incorporated into the ration to stimulate growth of the bird. Powdered skim-milk and buttermilk in certain formulas for chick rations seem to be the most flagrant offenders in this regard. Too many wheat middlings are under suspicion also. The cardinal problem in coccidiosis control is to construct a ration that is adequate in vitamin and vitamin-like materials for the normal development of the host, but at the same time lacks inordinate coccidium-stimulating properties. The third diet described above is a step in this direction.

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### THE FERMENTATION OF CIGAR-LEAF TOBACCO<sup>1</sup>

CONTRARY to the observations of Loew,<sup>2</sup> investigations at the Pennsylvania Agricultural Experiment Station emphasize the significance of microorganisms in the normal fermentation of cigar-leaf tobacco. An epiphytic flora, consisting chiefly of members of the genera *Bacillus*, *Staphylococcus*, *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor*, was found upon the cured leaf. During the fermentation, species of the genus *Bacillus* were found to multiply rapidly; agar plate counts of these organisms increased from values of less than 500,000 per gram to values in excess of 2,000,000,000 per gram. Direct counts revealed increases from an initial figure of less than 500,000,000 per gram to counts greater than 15,000,000,000 per gram. The rate of multiplication depended, among other factors, upon the quality of the substrate, the amount of moisture present and the temperature maintained. Although members of this genus were present in the spore state at the end of the curing process, chains of vegetative cells of this group appeared in the early stages of the subsequent fermentation. Two types take part in this extensive multiplication of spore-formers. One type was easily identified as *B. megatherium*. The latter organism represents either a peculiar variation of *B. subtilis*, or it may be classified as a previously undescribed species. The second organism is a slender, motile rod, forming central to

excentric spores. The response of this bacillus to biochemical tests corresponds closely to the reactions of *B. subtilis*. Colonies upon agar are spreading, adherent and somewhat mycoides-like.

*Staphylococci* were frequently observed upon cured and fermenting tobacco. Occasionally the numbers equaled or exceeded those of bacilli, but in many cases the normal fermentation progressed without the appearance of this organism; on the other hand, bacilli were always found in the fermenting tobacco.

Although present in significant numbers on cured tobacco, members of the genera *Aspergillus*, *Penicillium*, *Mucor* and related types were found to decrease in number during the course of the fermentation. It was found, however, that an acid-agar medium was of value in the study of cured and curing tobacco. The predominance of *Aspergilli* and *Penicillia* on these plates seemed to indicate a tobacco difficult to ferment. This, apparently, is associated with the degree of complexity of carbohydrate and nitrogenous material in the leaf.

Studies of the catalase activity of the tobacco revealed a direct relation between the number and the activities of the microorganisms on the leaf. Low bacterial counts were invariably accompanied by slight catalase activity and high counts by considerable catalase activity. Any experimental treatment of the leaf that resulted in the inhibition of bacterial growth prevented increases in catalase activity. Any increase in catalase activity was accompanied by an increase in bacterial numbers. Catalase activity was restored to tobacco rendered inactive by heat treatment when inoculated with cultures previously isolated from tobacco.

This work will be reported in detail in a series of technical bulletins of the Pennsylvania Agricultural Experiment Station.

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<sup>1</sup> Authorized for publication on July 29, 1937, as paper No. 781 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

<sup>2</sup> O. Loew, "Curing and Fermentation of Cigar-Leaf Tobacco," U. S. Dept. Agr. Rep't. 59, 1899; "Physiological Studies of Connecticut Leaf Tobacco," U. S. Dept. Agr. Rep't. 65, 1900; "Catalase, A New Enzyme of General Occurrence, with Special Reference to the Tobacco Plant," U. S. Dept. Agr. Rep't. 68, 1901.

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