These reactions of Japanese virus in animal species, while differing sharply from those of St. Louis virus, are indistinguishable from those of louping-ill virus.[7] Serologically, however, Japanese B and louping-ill viruses appear unrelated.

Present knowledge indicates, therefore, that St. Louis and Japanese B encephalitis viruses, and hence the two diseases, are distinct. Japanese B and louping-ill viruses, however, are very similar to each other and also to the virus of Australian X disease as described.[8] Accordingly, possible relationships among louping-ill encephalitis of sheep in Scotland, apparently transferable to man,[9] Japanese summer encephalitis of man and Australian X disease should be further explored.

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DIETARY CONTROL IN EXPERIMENTAL COCCIDIOSIS

THERE is no effective control known for coccidiosis except stringent sanitary measures based upon an understanding of the life cycle of the protozoon. For a number of years the writer has been investigating the possible relation of diet to certain aspects of coccidian infection, particularly in the white rat. Recently efforts have been made to construct rations for the chick that would ameliorate the severity of caecal coccidiosis occasioned by *Eimeria tenella,* which seems to be by far the most virulent of the types found in this bird. Only three of the most striking experiments will be reported here in brief, but a completer report of the experiments will appear later.

One lot of control chicks was kept on a commercial ration that it had received from time of hatching. The ration was 40 per cent. yellow corn-meal, 30 per cent. wheat flour middlings, 5 per cent. ground hulled oats, 5 per cent. powdered skim-milk, 5 per cent. wheat bran and 15 per cent. various amounts of salt, cod liver oil, oyster shell, meat and bone meal, alfalfa meal, bone meal and charcoal. Another lot was given the same mixture until it was fourteen days old, when it commenced to receive the following test mixture: yellow corn-meal, 25 parts; meat and bone meal, 6; soybean meal (with nutty flavor), 22.5; hulled oats, 20; ground wheat, 10; wheat bran, 5; ground oyster shell, 1; salt, 1; charcoal, 1; beet sugar, 6.5; cod liver oil, 2. After nine days on the test ration, both controls and tests were infected with 50,000 sporulated oocysts of *Eimeria tenella* per bird. As a result of this infection nine out of eighteen controls succumbed, but only one out of thirteen in the test series.

One lot of chicks was maintained throughout on the control ration previously described. Another lot received the same until it was eight days of age, when its diet was changed to the following, by parts: yellow corn-meal, 32; hulled oats, 20; soybean meal (the kind with nutty flavor), 16; fish meal, 65 per cent. protein, 4; meat and bone meal, 4; wheat bran, 5; wheat flour middlings, 10; oyster shell, 2; alfalfa meal, 2; commercial casein, 1; salt, 1; charcoal, 1; cod liver oil, 2. After the second lot had been on the test diet for fourteen days, each bird in both lots received 60,000 *Eimeria tenella* oocysts. As a direct result of this infection nineteen out of twenty-four control birds succumbed, but only one out of twenty-four in the test series.

The two preceding test rations were constructed with the idea of eliminating powdered skim-milk and butter-milk altogether from the ration and reducing the amount of wheat flour middlings well below that in the control diet. Certain previous experiments had led us to suspect that these materials in certain combinations, particularly the skim-milk and butter-milk, were the chief inciters of ill effects in infected chicks. The chicks made good gains on both test diets, but four on the second test diet developed a partial paralysis of the type in which the toes turn inwards. They recovered within two days when placed on the control ration again. Since skim-milk has been shown to be a preventative for this type of paralysis, and since the second ration was shown to be slightly deficient in the paralysis-causing factor, a ration was constructed in which a small amount of skim-milk was included and the grain element somewhat varied. It was, by parts, as follows: yellow corn-meal, 40; hulled oats, 10; ground whole oats, 5.5; barley, 5; ground whole wheat, 5; wheat bran, 5; wheat flour middlings, 5; soybean meal, 10.5; meat and bone meal, 4; fish meal (65 per cent. protein), 2; oyster shell, 2; salt, 1; skim-milk, 2; alfalfa meal, 3.

The chicks put on the latter ration at the age of thirteen days made excellent growth during the next fourteen days. At the end of this time twenty-eight chicks on the control ration and thirty-three on the test ration were each given a forced feeding of 80,000 sporulated oocysts of *Eimeria tenella.* As a result of this infection seventeen chicks on the commercial or control ration succumbed, or about 60.7 per cent. There were only six fatalities in the group on the test diet, a mortality of only 18.7 per cent. No paralysis appeared in this group.

These and other experiments by the writer prove that coccidiosis is a disease controllable through the diet. It appears that the extreme severity manifested

[7] W. A. Pool, *Proc. Roy. Soc. Med.*, 27: 707, 1934; E. W. Hurst, *Jour. Comp. Path.*, 44: 231, 1931.

[8] J. R. Perdrau, *Jour. Path. and Bact.*, 42: 59, 1936.

[9] T. M. Rivers and F. F. Schwentker, *Jour. Exp. Med.*, 59: 669, 1934.

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[1]

CONTRARY to the observations of Loew,[2] 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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[1] Authorized for publication on July 29, 1937, as paper No. 781 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] 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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