per cent. Across the road from this farm, where a 60 per cent. loss had been sustained in 1929–1930, weed eradication was practiced, and only 6 per cent. of the celery was thrown away on account of mosaic. In another field where eradication measures had been practiced, only 4 per cent. of the crop was lost. Weed eradication was not started around these two fields until ten days after the crop was transplanted. In another field weed eradication measures were put into effect two months after transplanting; and at harvest time the loss from mosaic, 25 per cent., was practically as severe as where no weeds had been removed.

Detailed laboratory and greenhouse studies, made during and following the winter of 1930–1931, showed that Commelina was probably the only important wild host and that the celery aphid was probably the only important insect carrier of the trouble. In 1931–1932 weed eradication was started before celery was transplanted and continued during the winter. Commelina was the only weed removed from about experimental plots. This winter was unusually warm and mosaic spread much more rapidly and widely than during the previous season.

Commelina was not removed from around one field during the winter of 1931–1932. At harvest a small number of plants still remained healthy, but the grower considered it too small to be worth harvesting. In two near-by fields around which Commelina had been removed, one showed that less than one per cent. of the crop was lost due to mosaic, and in the other the loss amounted to a little more than one and a half per cent.

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THE EFFECT OF THE ELECTROPURE PROCESS OF TREATING MILK UPON BACTERIAL ENDOSPORES[1]

IN a recent paper[2] the Electropure process was described, and it was concluded that the process operating at 71° C. on an experimental basis was very effective in destroying resistant bacterial endospores. The results of these experiments indicated that another factor, in addition to heat developed in the milk, might be partially responsible for the endospore destruction. There seemed to be a strong indication that, in the case of endospores, the destruction might be partially caused within the cells. It is a known fact that the more concentrated an electrolytic solution becomes, the less resistance it offers to an alternating current and the greater the amount of heat produced in consequence. The cytoplasm in the bacterial endospores becomes more concentrated due to loss of water, and consequently the electrolytic substances in solution within the cells offer less resistance to the electric current than does the surrounding medium (milk). As a result, an instantaneous and marked increase in temperature within the cells themselves is effected. The heat thus created is probably sufficiently intense to cause the destruction of the spores.

The idea brought out in the above statement has been demonstrated in the laboratory by immersing an artificial cell, consisting of a parchment sac, filled with a 1.8 per cent. NaCl solution, between two carbon electrodes in a vessel containing a 0.9 per cent. salt solution. A sensitive thermometer was suspended in each solution, respectively, and, when the current was applied (110 v, A. C.) the temperature of the solution in the outer vessel at the end of 0, 10, 20, 30, 40, 50 and 60 seconds was, respectively, 21°, 26°, 31°, 39°, 57°, 76° and 86° C.; while in the parchment sac it was 21°, 28°, 37°, 48°, 62°, 80° and 88° C. The medium in the parchment sac contained the greater amount of free ions and, therefore, offered the least resistance to the current, and as a result more current flowed through the cell, and consequently more heat was generated.

A similar experiment was conducted in which the salt solutions both in the suspended cell and the surrounding medium were of equal concentration (0.9 per cent. NaCl), and a very heavy suspension of *Bacillus megatherium* spores was added to the inner or suspended cell. The temperature in the outer vessel at the end of 0, 10, 20, 30, 40, 50 and 60 seconds was, respectively, 21°, 25°, 31°, 37°, 48°, 62° and 78° C.; while in the parchment sac, containing spores in addition to the salt solution, it was 21°, 25°, 31°, 39°, 59°, 80° and 98° C. The data given from the above two experiments are representative of repeated tests.

The results of the first experiment showed that though the rise in temperature in the suspended cell was much more rapid than in the surrounding medium, the temperatures in both liquids quickly tended to become equal, and both reached the boiling point at about the same time. This was probably due to the fact that diffusion through the type of sac used was very rapid. The results obtained with the spores, however, showed that though the temperature rise was practically equal in both the inner and outer liquids for the first 30 seconds, the rate of increase became

[1] Journal Article No. 118 (M.S.) from the Michigan Agricultural Experiment Station.

[2] A. J. Galpi, Jr., and E. D. Devereux, "Effect of the Electropure Process and of the Holding Method of Treating Milk upon Bacterial Endospores," *Jour. Dairy Science*, 13, 368–371. 1930.

markedly greater thereafter. It is possible that this increase in heat in the suspended cell could have been brought about by "radiation" from the heated spores in the liquid. If this was the case, then there is reason to believe that the temperature within the spores was probably much higher than in the surrounding medium, which would aid in the destruction of the endospores. This point could be brought out more clearly if the combined volumes of the spores in suspension were greater in proportion to the suspending liquid. A more striking difference in temperature would undoubtedly be evident, due to the increase in radiated heat.

SUMMARY

The results of these experiments seem to indicate that the destruction of endospores by this electrical process is not entirely due to the heat created in the medium surrounding the endospores but also by another heat factor, namely, the heat generated within the endospores. Also, the temperature attained within the endospores is probably greater than that of the surrounding medium.

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DISSOCIATION OF CL. WELCHII

PLATING out pure cultures of *Cl. welchii* on suitable media has given rise to the development of two distinctly different types of colonies, the one a smooth hemispherical mound with sharply defined margins, the "S," the other a flat granular colony with an irregular or flagellated margin, the "R." Repeated fishing of colonies characteristic of the two types results in the development of pure strains of the two; in the case of the "S," the cultures usually show a few colonies with an "R"-like outgrowth even after many generations, the "R" types on the other hand become fixed much more readily; in the case of one culture, the "R," fished directly from the primary plate from an old laboratory culture containing both types, bred true for many generations

These two types present the usual morphological appearance and give the characteristic reactions of *Cl. welchii* so far as carbohydrate fermentations and stormy fermentation of milk are concerned. In fluids the two types appear strikingly different in their habit of growth. The "S" produces an even turbidity with little tendency to develop a deposit, while in the case of the "R" there is a heavy bottom growth and almost clear supernatant. With the latter type, after several cultural generations in broth, a faint turbidity frequently appears and on plating out such a turbid culture, some "S" colonies always develop which may be established as a pure strain by repeated fishing of characteristic colonies. Acid agglutination reactions and cataphoresis studies indicate the iso-electric point of "S" suspensions to be much more acid than that of the "R" type. Agglutinins specific for the two types are produced by the treatment of rabbits with washed suspensions.

There is also a marked contrast in the pathogenicity of the two types. Pigeons die in approximately four hours, following the introduction into the breast muscle of one cubic centimeter of twenty-four hour fluid cultures of the "S" type, while the same amounts of "R" cultures, grown in the same manner, have very little apparent effect.

The "S" types produced haemotoxin from ten to twenty times more potent than that produced by the "R," as measured by *in vitro* haemolysis of red cells, although both "S" and "R" haemotoxins are neutralized by stock *Cl. welchii* antitoxin. As might be expected, the "S" toxins, when introduced intravenously in suitable doses into experimental animals, result in a rapidly developing profound anemia, and blood films made during the course of the anemia show a marked degree of anisocytosis, similar to that shown in earlier papers by the use of toxin from undissociated cultures of *Cl. welchii*. The "R" toxins in the same dosage have little effect. When given in doses correspondingly large, taking the haemolytic titer as the criterion, a definite anemia is produced, though not so marked as that which follows injection of the "S" toxin nor is the anisocytosis so conspicuous.

It appears that *Cl. welchii* behaves as the many aerobic species of bacteria which have been studied from this angle. A detailed paper is in process of publication.

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