A SIMPLE METHOD OF DETERMINING THE THERMAL DEATH-POINT.

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Methods of determining the thermal death-point of bacteria have been described by Novy[1], Sternberg[2], Smith[3], and many others. The apparatus ordinarily used is illustrated by Novy and by Smith. The standard procedure consists of a ten-minute exposure of a suspension of the bacteria in a capillary tube or test tube to the temperature desired by immersing the tube in a hot water bath. The methods vary as to the medium and the type of tube used for the suspension, the regulation of the temperature, and the mode of testing the viability of the organisms.

In recent studies of the thermal death-points of certain bacteria causing plant diseases, some modifications of the older methods have been adopted. A heavy suspension of each organism was made in a flask of sterilized distilled water, and equal amounts were then transferred with a sterilized pipette to small sterilized test tubes, one cm. in diameter and seven cm. in length, with walls of fairly uniform thickness. With plant pathogenes there seems to be no especial need of using bouillon or salt solution for these suspensions.

For the water bath a five-gallon wooden candy bucket was placed in a sink under a combination steam and cold water faucet from which a piece of hose led down into the bucket (fig. 1). By turning on either the cold water or the steam and stirring with a stick, the temperature of the water was easily adjusted and controlled. The temperature in such a container is more easily controlled than in the smaller metallic types because of the large volume of water and the relatively low thermal conductivity of the wood. If steam is not available, hot water can be used to raise the temperature since plant pathogenic bacteria are not spore-formers and have low thermal death-points. A. G. Johnson and others working at the University of Wisconsin have made use of a somewhat similar container.

The test tubes containing the organisms were inserted in holes bored in a large, flat, cork float about 15 cm. in diameter so as to project well down into the water. By means of a certified thermometer reading to one-tenth of a degree inserted through a cork into a similar test tube containing water and suspended at the center of the float, the exact temperature of the contents of the tubes could be controlled. The temperature of the water bath was adjusted before the cork float with the test tubes was placed on the surface. After the contents of the tubes had reached the temperature of the bath, a matter usually of about one minute, the float was allowed to remain ten minutes and then was

[1] Novy, Frederick G. Laboratory work in bacteriology. 1-563. 1899. p. 513.

[2] Sternberg, George M. A text-book of bacteriology. 1-708. 1901. p. 154.

[3] Smith, Erwin F. Bacteria in relation to plant diseases. *1*:1-285. 1905. p. 75.

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removed to a jar of cold water so as to cool the tubes at once. The tubes, properly labelled, were then removed to be tested and were replaced by another series of tubes to be exposed to the next higher temperature. Temperatures between 45° and 55°C. were thus tested. The viability of the organisms in each series of heated tubes was tested by means of loop transfers to tubes of slanted agar, and the suspensions

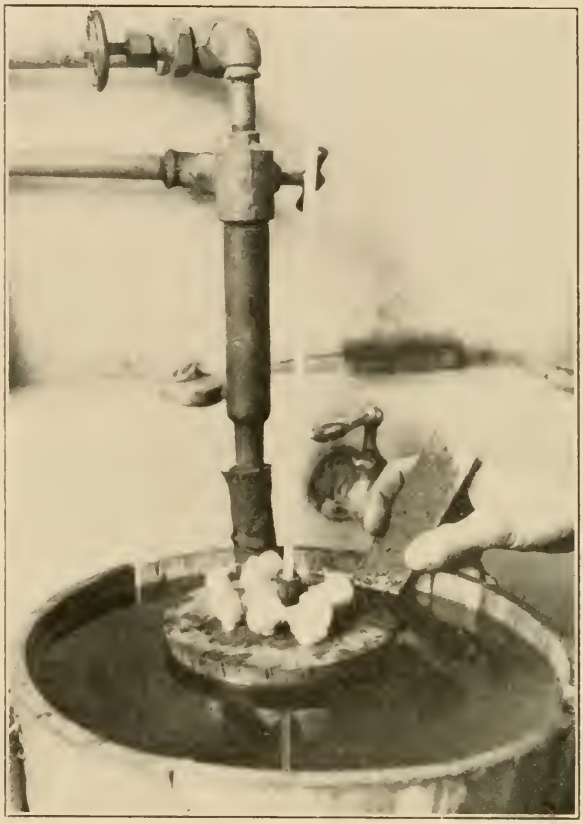

Fig. 1. Thermal death-point apparatus. Large cork float supports thermometer and a number of small test tubes containing the bacterial suspensions. Temperature of water bath adjusted by steam and cold water.

were preserved so that additional transfers might be made from those representing the critical temperatures.

To summarize, the essential phases of this method are the use of water suspensions in small test tubes, the use of a large, wooden container for the water bath in which the temperature is raised by the addition of steam or hot water, the use of a cork float to support the tubes and the thermometer, and the test for viability by making transfers to agar slants.