

Thermal Death Point of Streptococci*

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THE investigations forming the basis of this paper were undertaken because of the expressed wish of some of the members of the Association that a larger number of strains of pathogenic streptococci from varied sources be tested as to their thermal death point at 30 minutes' exposure, before a final decision be made as to the proper temperature necessary for commercial pasteurization.

In order that we may have fresh in mind the previous work on streptococci let us summarize the results of the rather recent elaborate investigation by Ayers.

In 1918, Ayers¹ studied a number of streptococci as to their thermal death point. Cultures of 5 were completely killed after an exposure of 30 minutes to a temperature of 140°, 12 at 135°, 5 at 130°, and 5 at 125° F. He also tested 6 non-hemolytic varieties of streptococci derived from pus from the pleura, scrotum, elbow joint or the blood. These were not killed when exposed for 30 minutes to 140° F. They required heating to temperatures as high as between 55° and 60° C. These are varieties, however, which are not known to have been communicated through milk to man. From Ayers and Johnson we also have the knowledge that among streptococci obtained from the cow resistant strains of streptococci are found. Forty-six of 139 such strains required an exposure of 30 minutes to temperatures of 71.1° C. (160° F.) to 73.9° C. (165° F.) to cause their death. These streptococci, however, so far as known, are not pathogenic to man.

Summing up the results of these investigations we find a small portion of the strains of hemolytic streptococci which may be transmitted by milk and are pathogenic to man, are more resistant than the majority and withstand heating to a temperature of 135° F. for 30 minutes, but not one to a temperature of 140° F. for 30 minutes. The remainder are killed at temperatures ranging from 125° to 135° F.

We now turn to the present investigations, the object of which is, if possible, to satisfy ourselves, by testing the thermal death point of a large number of hemolytic streptococci obtained from human infections, what degree of temperature is sufficient to destroy the most

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resistant in 30 minutes. Our present information leads us to believe that the diseases shown to have been transmitted through milk by means of streptococci have always been due to the hemolytic varieties.

The few non-hemolytic strains believed to have caused occasional human infections, have been through the taking of food products such as cheese. These so-called "green" streptococci would require much higher temperatures or much longer exposures, so that from the point of view of pasteurization it would be impracticable to safeguard against them, unless they were a very real danger. The streptococci utilized in our tests were 100 strains obtained from the following sources:

Source of Strains	Number of Strains
Cases of	Tested
Septic Sore Throat	20
Tonsillitis	20
Scarlet Fever	18
Mastoiditis	9
Glandular fever	7
Erysipelas	18
Other sources	8
Total hemolytic strains	100



METHODS USED IN TESTING THE THERMAL DEATH POINT

The First Method was to fill vials with about 10 c.c. of sterile milk and place them in a water bath, the water of which was somewhat higher than the desired temperature. Then the milk in the vials and the water in the water bath having reached the desired temperature, 0.05 c.c. of a blood broth culture of the strain of streptococcus to be tested was injected into the milk and the vial stopped by a rubber cork already heated to the same temperature. The stoppered bottle was then submerged in the heated water for the required times. It was then removed and the contents immediately cooled by pouring the contents into an ice-cold 100 c.c. bottle which was immediately packed for a few minutes in cracked ice. The bottle with its contents was then removed and placed in the incubator at 37.5° C. for approximately 42 hours. The milk in the vial was then tested for viable hemolytic streptococci on blood agar plates.

The individual strains were also tested in a slightly different way by filling the bottles with water and covering them while submerged with softened parchment. This made it possible to be certain that no air pocket was present. The culture of streptococci was injected after piercing the parchment with the needle of the syringe holding the culture.

The Second Method approached the actual pasteurizing process—250 c.c. of milk held in a bottle at the desired temperature was inocu-

lated, and then after a moment's shaking discharged into a funnel heated to the same degree, and through this into a lead coil submerged in water held at the desired temperature. At the proper time the submerged outlet of the coil was raised, the metal stopper removed, and a sample taken. The end was then plugged, plunged in boiling water, and dropped back until the next sample was taken. For this method a large number of strains were mixed together.

Further—through the kind coöperation of Dr. Bundesen—Dr. Tonney, Assistant Commissioner and Director of the Chicago Department of Health Laboratories, subjected 100 strains of hemolytic streptococci derived from similar cases in Chicago to tests carried out by the method of the submerged vials. The results of these tests will be given first.

Thermal death point in milk of 100 strains of hemolytic streptococci exposed at 20 and 30 minutes as reported by Dr. Tonney were as follows:

Sixty strains (sources—milk 10, epidemic sore throat 1, ears and throats of scarlet fever patients 10, throats from cases of contagious diseases 38, and University of Chicago Laboratory 2) were killed after an exposure of 30 minutes to 135° F. Of the remaining 40, there were 2 killed by an exposure to 138° F. for 30 minutes and 38 were killed after exposure to 140° F. for 30 minutes, but probably not killed after exposure of 20 minutes.

The sources of the more resistant 38 strains were—milk 4, epidemic sore throat 1, University of Chicago 1, contagious disease wards 32.

Dr. Tonney wrote me that he did not test the milk in the vials for hemolytic streptococci that seemed to have a growth after 10 and 20 minutes' exposure as his interest was mainly in the 30 minutes' exposure. As some of those which were exposed to 140° for 30 minutes and showed growth in the milk on testing were found to have no living hemolytic streptococci, it is probable that if the milk in the vials heated for 10 to 20 minutes had been tested, the growth might have been shown to be due to other bacteria.

Results obtained in New York City Laboratories were as follows:

Cultures of streptococci from every one of the 100 strains were killed after 10 minutes' exposure to 140° and after 30 minutes' exposure to 138° F. Thirty combined cultures in milk when passed through the lead coil were killed after 30 minutes' exposure at 136° or 20 minutes at 138° or 10 minutes at 140° F. The destruction of most of the streptococci was surprisingly rapid when heated to 138° F. in milk.

Thus, 25 mixed cultures of erysipelas strains added to milk, so that 1 c.c. contained about 10,000,000 streptococci showed no growth after

5 minutes' exposure, either in blood plates immediately inoculated with 1 c.c. or in plates inoculated from 3 c.c. of milk after 42 hours of incubation.

PATHOGENIC NON-HEMOLYTIC STRAINS OF STREPTOCOCCI

In the issue of August 6, 1926, of the *Public Health Reports*, Linden, Turner, and Thom report two outbreaks of food poisoning due to the eating of cheese. In all, 31 persons suffered from an attack of pains in stomach, nausea and diarrhea. Samples of the two lots of cheese showed enormous number of bacteria. These were mostly short non-hemolytic streptococci. Milk cultures of these streptococci caused illness when fed to cats.

They report that of 3,500,000 of these organisms subjected to 143° F. for 30 minutes, 6,100 survived, but that of 1,000,000 exposed to 145° F. for 30 minutes none survived. In our tests they have been more resistant.

In our laboratory Miss Oldenbusch subjected the culture to temperatures of 143° and 145° F. with the following results:

TIME OF EXPOSURE	AT	143° F.	145° F.
			Streptococci in 1 c.c.
30 minutes		4,000,000	2,500,000
45 "		1,950,000	450,000
60 "		1,440,000	48,000
75 "		14,000	700
90 "		900	0

The infected milk before heating when plated developed about 40,000,000 colonies per c.c.

There are at least two important reasons why the existence of these heat resistant non-hemolytic streptococci have little or no bearing on the question of the proper temperature to be used in the pasteurization of milk. The first is that no outbreak of milk infection has been traced to them, the second that the temperature required to kill them is so great that it would damage the taste and appearance of the milk to a degree which would make it much less desirable as a food.

I believe no one would suggest that pasteurization of milk be carried on for 90 minutes at a temperature of 147° to 148° F.—the two degrees being necessary as a margin of safety.

THERMAL DEATH POINT OF BRUCELLA ABORTUS

The importance of infections with this microorganism having been so recently appreciated, little work has been reported upon its resistance to heat. The results of the following experiment are given so as to complete the study of the thermal death point of pathogenic bacteria transmitted through milk. These tests were carried out in the New York City Department of Health Laboratory by Carolyn Oldenbusch.

Sterile milk was bottled in 9 c.c. amounts and heated to a temperature of 1° C. above test temperature. One c.c. of a suspension of 13 strains of *B. melitensis* and *B. abortus* (of human, bovine and swine origin) was added to each bottle which was then submerged in a water bath at the appropriate temperatures, for the desired length of time.

On removing from the water bath, bottles were plunged into cracked ice and quickly and thoroughly chilled. Four plates were then made from the milk, and again after the milk had been incubated for 48 hours at 37° C. Controls showed there were 5,000 billion organisms per c.c. of milk.

At 140° F. the cultures survived 7½ minutes, but were killed at 10 minutes. At 142° F. they survived 5 minutes and were dead at 7½ minutes, while at 145° F. the milk was sterile at the end of 5 minutes' heating.

SUMMARY

Two hundred strains of hemolytic streptococci obtained from different cases of septic sore throat, scarlet fever, erysipelas and other diseases known to be due to hemolytic streptococci and transmitted at times through milk were tested as to their thermal death point while in milk after 30 minutes' exposure.

One-half of these were examined in the laboratories of the Chicago Department of Health under the direction of Dr. Tonney and one-half in the laboratories of the New York City Department of Health.

Cultures from every one of the 200 strains were killed by an exposure to 140° F. for 30 minutes. The majority of strains were killed at a temperature of 136° F. or less.

The resistant, probably pathogenic, non-hemolytic streptococcus found in cheese was not killed after 75 minutes' exposure to 145° F. but was killed after 90 minutes.

Cultures of *B. melitensis* and *B. abortus* were killed after 10 minutes' exposure at 140° F.

REFERENCE

1. Ayers. Thermal Death Point of Pathogenic Streptococci. *J. Infect. Dis.* 23:290, 1918.