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Significance of Thermophilic Bacteria in Pasteurized Milk*

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IN discussing this subject we feel that it is best to attempt to answer certain questions which probably exist, or have existed at some time, in the mind of everyone concerned with the control of city milk.

What are thermophilic bacteria? It may not be necessary to go into detail, but it is well to define rather exactly what we propose to discuss. We shall include not only the obligate thermophiles which grow only at temperatures above the usual limits of bacterial growth but also the facultative thermophiles which grow normally at ordinary temperatures and are able to multiply at 50° C. or higher.

There are no natural limits for these groups. The obligate thermophiles have a temperature range running as high as 85° C. Some facultative thermophiles grow as low as 20° C. and as high as 60° C. We are interested in those which grow at the usual pasteurizing temperatures and those which are unable to grow at this temperature but grow readily at a few degrees below this point.

The upper thermal limit of the growth of any particular strain of bacteria is much more sharply defined than the lower. Bacteria which are inhibited or destroyed at 145° F. may grow rapidly at 140° F.

The thermo-resistant bacteria which, although they do not form spores, survive pasteurization, should not be confused with the thermophiles. Both groups of bacteria sometimes produce pinpoint colonies on agar plates but otherwise there is no connection between them.

There has been a tendency to look upon the thermophiles as a natural group of related bacteria. On the contrary it is a hetero-

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geneous collection including representatives of many unrelated species. They should be regarded as variants which may occur in any species and which usually differ from the normal only in their ability to grow at high temperatures.

We find among the thermophiles representatives of the streptococci, the lactobacilli, the colon group, anaerobic, and especially aerobic spore-forming rods. The last is the group most commonly found in pasteurized milk.

What is the source of thermophiles in milk? They occur naturally under conditions which make it easy to account for their presence in milk. Thermophilic bacteria were first observed in thermal springs where they sometimes grow at temperatures over 80° C. They are known to occur in fermenting manure and other vegetable material. It would probably be possible to isolate thermophiles from almost any sample of cow manure. From these sources they become widely distributed and may be found in soil, dust, on the leaves of plants, and the surface of any exposed material.

Under some conditions the surface of the soil may reach temperatures high enough to favor the growth of thermophiles and it is possible that under some circumstances they may grow in the soil at relatively low temperatures.

We consider it very improbable that bacteria of this type ever actually live in the udder. It is true that milk drawn direct from the udder into test tubes and held at 50 or 55° C. will sometimes reduce litmus, but it does not necessarily follow that this milk is inoculated with thermophiles from the ducts of the udder. Very rigid precautions are necessary positively to exclude outside contamination. However, there are many things beside bacteria which reduce methylene blue and this reaction cannot be accepted as proof of bacterial growth without proper checks.

We have examined the udders of a large number of cows without securing any positive evidence of the presence of thermophilic bacteria. However, it is not necessary to assume that these bacteria grow in the udder to account for their almost universal presence in mixed herd milk. It is probable that almost any sample of mixed milk from the ordinary farm if held at 50–55° C. would show development of thermophiles.

Under what conditions do thermophiles attain high numbers in pasteurized milk? There are a number of conditions which may work singly or jointly to produce this result. One is an exceptionally high initial contamination before pasteurization. The milk from some individual farm may come to the pasteurizing plant heavily infected

with thermophiles. This is not common but in one plant investigated it was found that the milk from one farm gave a nearly pure culture of thermophilic bacteria.

The milk may be inoculated before pasteurization by adding to the batch milk returned from the wagons. If, as frequently happens, the end of the previous day's run was high in thermophiles, this may serve as an inoculation for the new run.

The milk may be inoculated from defective equipment, such as leaks which permit milk to stand in the lining of the vat and subsequently, when highly charged with thermophiles, to seep out into the pasteurizer.

The milk may also become infected from the foam and residue left from the previous batch. Thermophiles may also multiply in the pasteurizer when some part of the milk is not maintained at the proper temperature. The reduction of the pasteurizing temperature even a few degrees brings the milk within the growth range of many varieties which cannot grow at 145° F. This may happen in dead ends of pipes, possibly in corners of vats not reached by the agitators, and especially in the foam. It is well known that the foam is several degrees lower in temperature than the milk under it and not only do the more resistant bacteria carried by the foam survive, but it acts as an incubator for many types of thermophilic bacteria.

The most common cause of high thermophilic counts is the building up in numbers when successive batches are pasteurized in the same vat without adequate cleaning between runs. There is some

TABLE I

INCREASE IN THERMOPHILIC BACTERIA IN PASTEURIZER VATS

Vat No.	Run	Reduction Time at 55° C. Before Past. Hrs.: Min.	Reduction Time at 55° C. After Past. Hrs.: Min.	Direct Counts of Streptobacilli Before Past.	Direct Counts of Streptobacilli After Past.
I	1	8:41	8:01	20,000	20,000
	2	6:59	4:38	42,000	345,000
	3	6:09	1:18	121,000	4,500,000
	4	2:58	0:18	1,850,000	22,000,000
II	1	1:57	0:33	1,420,000	15,500,000
	2	2:34	0:48	515,000	8,760,000
	3	6:10 (Neg.)	1:26	30,000	4,850,000
	4	4:21	0:33	970,000	14,000,000
III	1	10:00 (Neg.)	9:54	0	0
	2	2:53	0:31	1,000,000	8,500,000
	3	3:47	0:50	130,000	12,600,000
	4	4:13	0:26	324,000	13,600,000
	5*	4:20 (Neg.)	3:33 (Neg.)	173,000	348,000

* Milk held in vat for 50 min. at 143° C.

multiplication in the first batch, started by the thermophiles which are almost invariably present in mixed milk. The foam and milk left in the bottom of the vat serve as an inoculation for the following run so that if five or six batches of milk are pasteurized in one vat the later ones are likely to have a high count. In Table I is shown how the bacteria which grow at high temperatures may grow in the vats and build up in numbers by the end of the last run of the day. The experiments were conducted in a large milk plant under normal plant conditions.

An examination of the results in Vat I shows a smooth and regular building up of thermophiles from very few after the first run to 22,000,000 at the end of the fourth. It will be noted that the reduction times correspond very well with the numbers of bacteria.

Vat II is an example of apparently bad contamination with thermophiles before the first run. The vat had evidently not been properly cleaned and scalded before the beginning of the day's run. This heavy initial contamination was reduced to some extent after several successive flushings with vats of milk but had started to build up again by the end of the day as is evidenced by the increase to 14,000,000 at the end of the fourth run.

Vat III shows a building up process for the first four runs of the day when the normal procedure was being carried out in the pasteurization of the milk. At the time of the fifth run, however, there was a break-down in the bottler and the milk had to be held for a longer time at 143° F. before the vat could be drained onto the cooler. In this case the milk was held over 50 minutes at pasteurizing temperature. The result was a marked decrease in the number of high temperature bacteria: from 13,600,000 at the end of the fourth run to 348,000 at the end of the fifth. This would seem to indicate that these particular bacteria were not favored by the pasteurization temperature and when the longer period allowed the foam, splash, etc., to attain a higher temperature their numbers were materially reduced. This is in keeping with results obtained by one of us on thermophilic bacteria from this same milk plant at another time. It was found that the bacteria increased rapidly in the foam which was at a lower temperature than the milk, but decreased rapidly in the milk or in foam brought up to the pasteurizing temperature.

What does the thermophilic count indicate? It indicates that one or more of the conditions which we have enumerated exist in the pasteurizing plant. The plant may be receiving unusual contamination; it may be repasteurizing milk brought back from the wagons; the walls of the pasteurizer may have a thin layer of cooked milk in which

thermophiles may grow; the construction of the pasteurizer may be defective so that some of the milk fails to reach the pasteurizing temperature; there may be an excessive amount of foam which is not heated to temperature; or the vats may not be cleaned between runs.

The value of the thermophilic count to the control officer is much the same as the value of the ordinary count. We do not count the bacteria in pasteurized milk because we are especially concerned with the destruction of the particular types of bacteria whose colonies grow on an agar plate. We make these counts because by them we can tell with reasonable certainty whether the milk has been heated to a temperature which will render it free from pathogenic bacteria; whether the plant is operated in a clean and efficient manner; and whether the milk is properly cooled between pasteurization and delivery.

There is no reason to think that the thermophilic bacteria are more harmful in milk than their relatives growing at lower temperatures. In fact since most of them grow very slowly or not at all at low temperatures there is a tendency to regard the thermophilic count as of no significance. Some have even advised to use culture media on which their colonies will not grow and to pretend that they do not exist. This ostrich-like attitude does not appeal to bacteriologists who believe that the plate count should be made to show as correctly and completely as possible the conditions which exist in the milk.

A control officer should welcome a technic which will enable him to detect those pasteurizing plants which are defective and make it possible for milk to leak into the walls of the vat, or which do not heat all of the milk to the required temperature. To be assured that between runs the vats are properly washed and steamed may not be a matter of major importance, but if the omission of this serves to obscure more serious defects it should be corrected.

On the whole we believe that an estimation of the number of thermophiles should be made of real service in the control of pasteurization. However, it is not sufficient to tell a dealer that his milk contains a large number of thermophilic bacteria and that this number must be reduced. Ordinary bacteria are a deep enough mystery for the average milkman. The inspector should be able to point out wherein his methods are defective and how they may be remedied.