

trical stimulation of the posterior ends of the ganglia was very striking in its magnitude and consistency of appearance. The average depression amounted to over 80% of the resting oxygen intake. A similar effect, but in a lesser degree, was observed when shocks were applied to the anterior ends of the ganglia. There was a prompt return to the resting rate when stimulation ceased.

Stimulation, at the rate of 2 shocks per second, of the ganglion of an isolated *Limulus* heart preparation was found by Asher and Garrey⁴ to produce definite depression of the heart rate, and this was confirmed by Garrey and Knowlton,⁵ who also found that stimulation at a faster rate produced complete inhibition of the entire ganglion. Therefore it is probable that the fall in oxygen consumption which we observed was due to inhibition. This is in harmony with Garrey's conclusion¹ that inhibition involves a decrease in cellular metabolism.

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A Life Cycle of a Thermophilic Organism.

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Numerous investigators have described organisms capable of growing at 62°C. This is the accepted pasteurization temperature for milk. In all of the descriptions of such thermophilic phenomena, especially where milk is concerned, one characteristic has been quite apparent—that is, sharp rather than gradual increases in bacterial numbers. Our observations have been in accord with those described in the literature. Calculation of the generation time for these increases has disclosed values of 5 to 8 minutes. It was thought that such short generation times were in little accord with accepted views. The only alternative was to consider the phenomena as evidence of something other than growth. Spores are usually present at the same time vegetative cells appear in smears of milk. It has been assumed that these spores are germinating rather than that the vegetative cells are sporulating. If spores exist in milk which shows thermophilic growth they must be present in large numbers.

⁴ Asher, L., and Garrey, W. E., *Am. J. Physiol.*, 1930, **94**, 619.

⁵ Garrey, W. E., and Knowlton, F. P., (personal communication).

In our experiments, milk diluted to 1 part to 100,000,000 disclosed micro-organisms in the very highest dilutions. The protocol of such an experiment is given in Table I. It will be noted that

TABLE I.
Showing effect of diluting milk containing thermophiles to dilutions of 100,000,000.
Note absence of growth in dilutions 5 and 6.

Temp.	Plate	1	2	3	4	5	6	7	8
30	1300	+	+	+	+	—	—	+	—
37	800	+	+	+	cocci	—	—	—	+
60	0	+	+	—	cocci	—	—	—	rods
		rods	rods		—				—
Transfers	+	+	+	+	—	—	+		
				rods			rods		

Hourly direct counts 0 up to 11 hours. 10,000,000—12 hour. Generation time 7.5 minutes.

there is a change of flora between the fourth dilution and the seventh, leaving a gap of 2 dilutions. This gap is rather characteristic of our experiments.

Continuous observations of this character have led us to the belief that there is a definite life cycle in the organism which we have been studying. It would be interesting if similar cycles were shown to exist for other organisms. Briefly, the cycle is as follows:

1. An invisible spore; present in large numbers, heat resistant, incapable of growth on ordinary laboratory media either at 37°C. or at 60°C.

2. A vegetative cell resulting from germination of (1); easily destroyed by heat, capable of growth upon ordinary laboratory media.

3. A spore forming within the rod (2); heat resistant.

4. A spore (3); devoid of all stainable material, heat resistant, incapable of growth on ordinary laboratory media.

5. A spore; invisible, heat resistant, not capable of growth upon ordinary laboratory media, dormant. This dormancy is broken by heat, cold, chemicals, such as alkali and acids.

Changing the organism from stage 5 to stage 1 makes it capable of germination, thus starting the cycle over again. A modification of this theory would permit the sequence of events to be stage 1, stage 3, stage 2, and so on. In our experiences such a theory more nearly explains many of the anomalous results reported in the literature, especially where thermophilic organisms in milk are concerned.