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Improved Temperature-Gradient Incubator and the Maximal Growth Temperature and Heat Resistance of *Salmonella*

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ABSTRACT

ELLIOTT, R. PAUL (Western Regional Research Laboratory, U.S. Department of Agriculture, Albany, Calif.), AND PATRICIA K. HEINIGER. Improved temperature-gradient incubator and the maximal growth temperature and heat resistance of *Salmonella*. Appl. Microbiol. **13**:73–76. 1965.—An improved all-metal temperature-gradient incubator produces its gradient by means of a bar permanently installed in a near-vertical position with its lower end in a cool constant-temperature water bath and with thermostatically controlled heaters near its top. Bolts hold the incubator in contact with the temperature-gradient bar, and polyurethane foam insulates the entire assemblage during use. Maximal growth temperatures of 34 representative strains of *Salmonella* were found to be between 43.2 and 46.2 C. In an agar medium with an initial level of 10^6 cells per milliliter, no strain survived 50 C for 48 hr. *S. senftenberg* 775W showed no greater heat resistance at or near 48 C than did other species or other *S. senftenberg* strains. However, it was considerably more resistant than other strains at 55 C.

An earlier temperature-gradient incubator (TGI; Elliott, 1963) gave good results but was heavy and awkward. Also, it required a pump and insulated tubing at the cold end, and thermocouples were difficult to install from beneath the troughs. This report describes a smaller all-metal TGI that gives equally good results and is much more convenient and durable. A series of tests on the maximal growth temperatures and heat resistance of *Salmonella* strains has proven the value of the improved TGI.

MATERIALS AND METHODS

Description of the temperature-gradient incubator (TGI). Figure 1 shows the assembly of the improved TGI. Its temperature gradient was produced by heat conduction from a bar (A-1, G-1) set in a fixed position with its lower end in a cool constant-temperature water bath (A-6), and its upper end heated under control by a thermostat (G-17, H-17). To keep the temperature gradient linear, the entire assemblage was insulated with polyurethane foam (A-2).

All parts of the incubator were aluminum alloy 2024-T4 except bolts and nuts, which were 0.25-in. stainless steel. The temperature-gradient bar (A-1, G-1) was 2 × 8 × 48 in., with holes bored for heaters and thermostats. Chromalox cartridge heating elements (H-17) (0.5 × 8 in., 400 w each, rated at 240 v; Edwin L. Wiegand Co., Pittsburgh, Pa.) received current through an adjustable transformer from a mercury-to-mercury relay controlled by either an adjustable- or a fixed-temperature mercury thermostat (Philadelphia Scientific Glass Co., Perkasie, Pa.) placed in either of the two holes (G-16, H-16) near the heaters. Heaters and thermostats fit their holes without excessive play. Pivoted bolts (G-15, H-15) at the side clamped the trough bar (E) firmly to the temperature-gradient bar (G) during use. A 0.125-in. aluminum cleat (G-5) positioned and supported the trough bar (E). The bottom ends of both bars were in the water during incubation. When the equipment was used for low-temperature studies, the water contained a suitable antifreeze.

The trough bar (E) was 1 × 8 × 36 in. It contained nine 0.5 × 0.5 × 28-in. troughs (E-11) labeled A to I and scaled 0 to 70 cm along their length. Troughs A and I contained thermocouples (D-12) at the 0, 34.5, and 70 cm marks. Barriers (B-10, C-10) were hermetically sealed with epoxy resin (Epon adhesive 934, Shell Chemical Co., New York, N.Y.) to prevent gas exchange between adjacent compartments. The same epoxy resin held the 0.25-in. aluminum sides (E-13) to the trough bar. Silicone rubber gaskets (B,9, C-9, D-9, H-9) (Durometer No. 55, 0.063 in. thick) topped the barriers and sides. Silicone rubber adhesive (RTV

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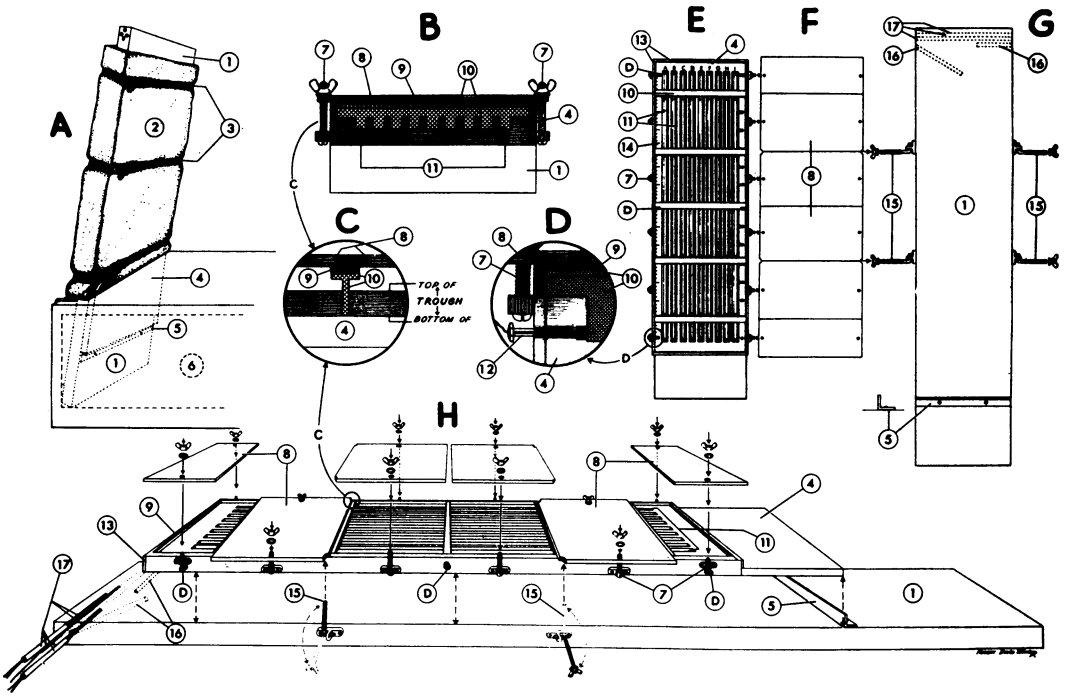

FIG. 1. *Improved temperature-gradient incubator (TGI). A. Position of TGI in use: (1) temperature-gradient bar; (2) soft polyurethane-foam insulation; (3) cloth straps (six) with buckles; (4) trough bar; (5) cleat; (6) constant-temperature water bath. B. Section through vapor barrier: (7) bolts and wing nuts holding lids; (8) lids (six); (9) silicone-rubber gasket; (10) aluminum vapor barriers (five); (11) troughs (nine). C. Section through trough bar and barrier. D. Thermocouple detail: (12) bolts bored for thermocouples. E. Top view of trough bar: (13) sides; (14) centimeter scale. F. Top view of lids. G. Top view of temperature-gradient bar: (15) pivoted bolts; (16) holes bored for thermostats; (17) holes bored for cartridge heaters (three). H. Exploded view of the TGI.*

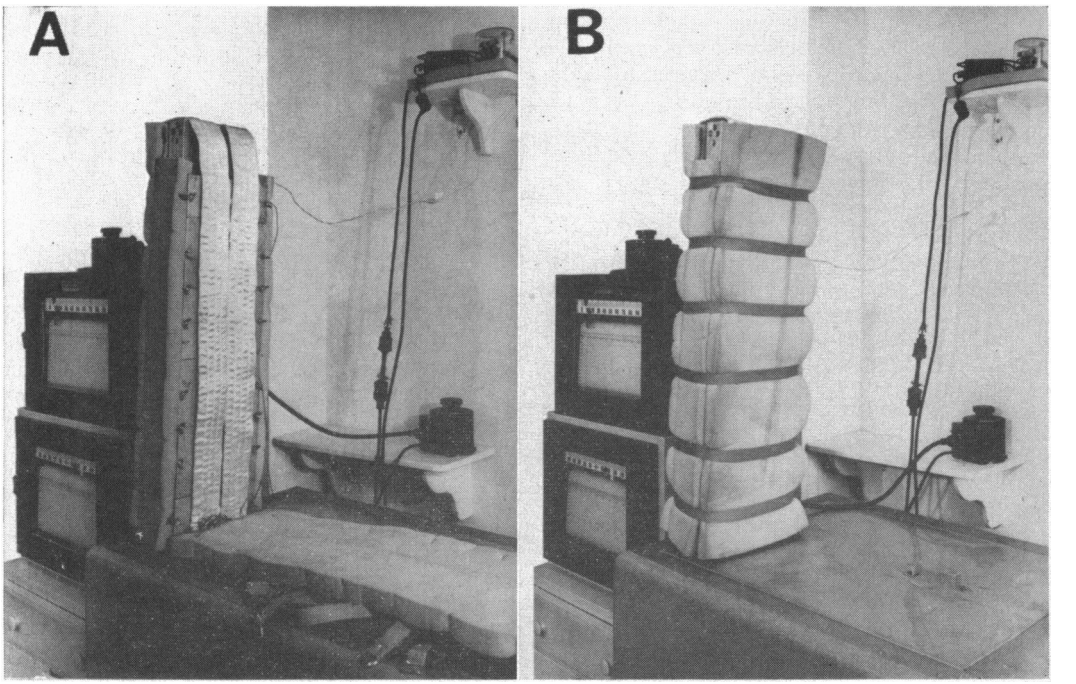

FIG. 2. *Temperature-gradient incubator. A. Assembled showing heating tapes for warming lids. B. During incubation.*

TABLE 1. *Maximal 48-hr growth temperatures and lethal incubation temperatures of Salmonella strains as determined on the temperature-gradient incubator**

Species	Strain	Maximal growth temp after 48 hr on gradient	Lethal incubation temp after 2 to 3 additional days at 28 C
		C	C
Group B			
S. typhimurium	TM1	45.5, 45.6, 45.7	
S. derby	5420	44.0, 44.4, 45.1	
Group C_1			
S. oranienburg	200E	45.6, 45.8	
	200 ESM	45.2	
S. bareilly	276	46.2	
Group C_2			
S. newport	5761	45.4	48.7
		45.8†	48.1†
		45.4, 45.4	
Group D			
S. dublin	1917	44.2	46.0
		44.6	47.6
		45.2†	47.2†
		45.4	
S. dublin	S1	44.5	
	S2	44.6	49.0
S. pullorum	Bc15h	44.8	45.0
	1431	44.4, 45.2	
S. gallinarum	3040	45.1	47.1
	5173	45.3	
	355	43.5	47.8
	4400	43.2	47.5
	4411	43.2	47.6
Group E_1			
S. anatum	6478	45.0	
S. meleagridis	5288	45.2, 45.4, 45.4†	
Group E_4			
S. senftenberg	775W	45.2	48.0
		44.5	49.4
		45.5†	48.1†
		45.3†	48.0†
		45.0, 45.6, 46.2, 46.2	
	3252	45.7†	48.1†
		45.7	47.9
		45.6	
Group G			
S. worthington	4661	45.0	
Other Groups			
S. memphis		44.9	47.6
		44.3†	47.0†
		44.8	

TABLE 2. *Maximal growth temperatures and lethal incubation temperatures of Salmonella senftenberg strains* as determined on the temperature-gradient incubator*

Strain no.	Maximal growth temp after 48 hr on gradient	Lethal incubation temp after 2 to 3 additional days at 28 C
	C	C
775W	45.8†	48.3†
3252	45.7†	48.0†
3652	45.0	45.5
4994	45.0	47.8
3167	44.9	48.0
21	45.2	47.6
4925	45.0	46.7
292	45.0	47.8
4466	45.3	47.7
4498	45.0	47.3
4952	44.9	48.0

* Strain 775W was originally isolated from eggs by Winter et al. (1946). The remaining strains were obtained from W. H. Ewing, Communicable Disease Center, U.S. Public Health Service, Atlanta, Ga.

† Average. Full data on strains 775W and 3252 are given in Table 1.

TABLE 3. *D** *values of Salmonella senftenberg 775W and S. typhimurium TM1 at 48 and 55 C in Trypticase Soy Broth*

Strain	D values at	
	48 C	55 C
S. senftenberg 775W	270	24
S. typhimurium TM1	300	8.5

* *D* = time in minutes for 90% of the bacteria to die.

102, General Electric Co., Silicone Products Dept., Waterford, N.Y.) held the gaskets in place.

Each compartment had its own lid (F), 9.5 in. wide to allow a 0.5-in. overhang on each side. Lids met at the barriers, and wing-nuts and bolts (B-7, D-7) held them in place. The air space between agar and lids was about 0.5 in.

Use of the incubator. The trough bar was closed loosely with the wing-nuts, then autoclaved in inverted position. The autoclave pressure was released rapidly, to permit the interior of the troughs to dry. When the bar was cool, inoculated medium containing 2% agar was poured into the

* Except as otherwise noted, inocula were 48-hr 35 C cultures in Trypticase Soy Broth added to melted and cooled Trypticase Soy medium containing 2% agar, at 10^6 bacteria per ml of agar. Replicate figures under each species are determinations made at different times. We determined maxima visually under bright light.

† Inoculum stored at 4 C in 0.85% NaCl for 7 days before use.

troughs in successively opened chambers. Then the lids were closed tightly and the trough bar was attached to the partly insulated temperature-gradient bar, in which a suitable temperature gradient had been established.

Slightly warm lids inhibit the tendency for the agar to dry out. If the incubation was above ambient, two wide heating tapes (Briskeat flexible heating tapes, 768 w each, Briscoe Manufacturing Co., Columbus, Ohio) were tied to the outer surface of the lids, and a low current was passed through them during incubation (Fig. 2A). In our experience, 1.5 amp of current sufficed. Heating tapes were unnecessary when incubation was below ambient. The assembly (Fig. 2B) was insulated and thermocouples were attached to a suitable recorder. It was necessary to use only two of the three thermocouples on one side at any one time because the gradient was not measurably different from linear.

In preliminary tests, the nine troughs of the incubator gave identical maximal growth temperatures with parallel 10^6/ml inocula of 48-hr suspensions of *Salmonella senftenberg* 775W (46.2 C) and of *S. typhimurium* TM1 (45.5 C). Only troughs A and I contained thermocouples, but good agreement in results indicated an identical temperature gradient in all troughs.

To determine lethal incubation temperature, the trough block was removed from the gradient bar and incubated at an appropriate temperature, as previously described (Elliott, 1963).

Results and Discussion

Table 1 shows maximal growth temperatures and lethal incubation temperatures for representative strains of *Salmonella*. All 48-hr maximal growth temperatures fell between 43.2 and 46.7 C, and no strain at 10^6/ml survived 50 C for 48 hr. *S. senftenberg* 775W showed no greater heat resistance on the gradient than did other strains (Table 2), although it is known to be several times more resistant than other salmonellae at temperatures higher than 50 C (Winter et al., 1946; Anellis, Lubas, and Rayman, 1954).

Experiments in which a water bath was used confirmed the finding that *S. senftenberg* 775W showed little heat resistance near its maximal growth temperature. We tested this strain in parallel with *S. typhimurium* TM1 at two temperatures by inoculating a 48-hr culture of each in Trypticase Soy Broth (TSB; BBL) into fresh TSB at 10^6/ml, then heating it to the test temperature quickly in a preheated flask. After 5 min for temperature equilibration, we took samples at intervals, plated them on Trypticase Soy Agar, and incubated them at 35 C.

Results (Table 3) confirmed that the two organisms are equally heat-sensitive at 48 C, but that *S. typhimurium* TM1 was much more sensitive than *S. senftenberg* 775W at 55 C. From the data in Table 3, we calculated Z_D values to be 12.0 for *S. senftenberg* 775W and 8.0 for *S. typhimurium* TM1. These values agree with those found by Angelotti, Foter, and Lewis (1960) for *S. senftenberg* 775W (Z_D = 11.83) and *S. manhattan* (Z_D = 8.95) heated in chicken à la king. Z_D values are the degrees F required for the decimal reduction time (D) to change 10-fold. D, in turn, is the time in minutes for a 90% kill (Anellis et al., 1954).

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