RUPTURE OF BACTERIA BY EXPLOSIVE DECOMPRESSION

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ABSTRACT

FOSTER, JOHN W. (University of Georgia, Athens), ROBERT M. COWAN, AND TED A. MAAG. Rupture of bacteria by explosive decompression. J. Bacteriol. **83**:330-334. 1962.—A device is described for instantaneously rupturing bacteria and other cells in a closed system under controlled conditions by explosive decompression. With this device, 31 to 59% of *Serratia marcescens*, ranging up to 20 mg (dry wt) of cells per ml, were ruptured after nitrogen saturation at 1740 psi. Under similar conditions, 10 to 25% of *Brucella abortus* and *Staphylococcus aureus* were ruptured. Rupture of these organisms produced readily separable cell walls. Centrifugation in linear glycerol gradients was applied to further separate cell walls from debris. *Mycoplasma gallinarum*, *Leptospira pomona*, and *Eimeria tenella* (avian coccidia) oöcysts were also broken up by the decompression chamber. Pressure and duration of saturation of cells with gas affected rupture efficiency. Within the limits of this study, concentration of organisms and volume of suspensions did not have a definite effect.

Fraser (1951) burst the bacterial cell wall and released the internal contents by sudden decompression. In his method bacterial suspensions were placed in a small gas cylinder, gas was added under pressure, and the suspension was shaken to completely saturate the organisms with gas. A valve was then opened and the suspension expelled into a beaker. The method employed by Fraser is not readily adaptable to the rupture of certain pathogens and does not offer instantaneous decompression. This paper describes an apparatus for instantaneous rupture of bacteria and other cells in a closed system under controlled conditions by explosive decompression.

MATERIALS AND METHODS

Preparation of cultures. Organisms used in this study were obtained from departmental stock cultures maintained by periodic subculturing. Mass cultures were grown on Difco tryptose agar in Blake bottles at 35 C. Incubation time was 16 hr for *Staphylococcus aureus* and *Serratia marcescens* and 36 hr for *Brucella abortus*. Growth was loosened with the aid of glass beads and washed off with 15 ml of cold (0 to 6 C) saline per bottle. Cell suspensions were filtered through glass wool in a Büchner funnel and centrifuged at 2700 × *g* for 30 min at 0 C in a Servall SS-1 head. The sediment was reconstituted to the original volume with cold saline, a sample removed for packed cell volume (PCV) determination, and the remainder centrifuged at 12,100 × *g* for 14 min at 0 C. After draining the sediment free of supernatant fluid, the cells were resuspended in cold water to the volume calculated to give the desired PCV. The PCV was determined in mm^3 by centrifuging in a Servall SP/X head at 1200 × *g* for 30 min at room temperature. A special heavy-walled tube was designed by one of us (T. M.) for determining PCV in an angle-head centrifuge. Suspensions of freshly grown organisms supplied by other staff members at the University of Georgia were also tested for rupture. The organisms and suppliers were *Leptospira pomona*, W. P. Van-Eseltine; *Mycoplasma gallinarum*, Frank Boyd; and *Eimeria tenella*, S. S. Sharma.

Description of apparatus. The apparatus (Fig. 1) consisted of a saturation chamber separated from a rupture chamber by a frangible disc. The frangible disc was held in a union-type safety head which gave a leak proof seal after the union was tightened. Saturation of cell suspensions with gas was accomplished with commercially available nitrogen (other gases can be used) through the aeration tube (5, 6) which connected to the gas source (1). A needle valve (2) permitted close regulation of pressure. A second valve (4) was placed so that the pressure gauge (3) could be cut off from the system during sterilization. Flanges (12, 15) were incorporated to facilitate disassembly for cleaning of the apparatus. A seal was accomplished by a teflon gasket (14) com-

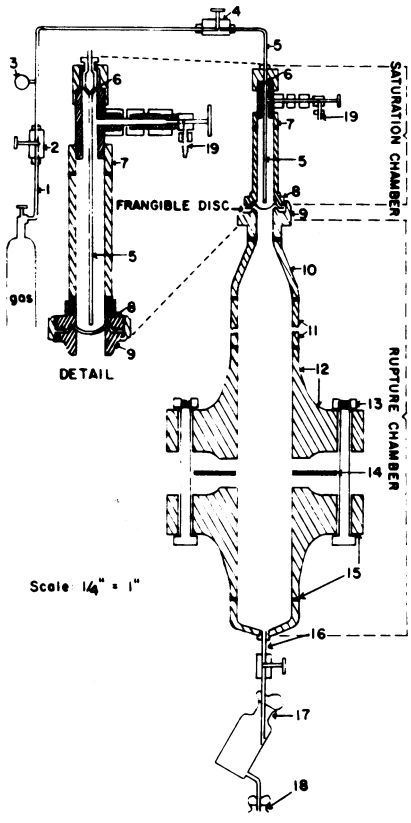

FIG. 1. *Diagram of decompression chamber. List of parts: Parts are of stainless steel except as noted. The saturation chamber is rated at 3000 psi. Rupture chamber is rated at 2500 psi. Dark bands represent welds.* (1) *Tubing, ¼ in. (outside diameter); Swagelok or Ferulok fittings used for connections;* (2) *Hoke needle valve (brass);* (3) *pressure gauge, 0 to 3,600 psi (brass);* (4) *cut-off valve for sterilization;* (5) *aeration tube to bottom of saturation chamber;* (6) *aeration tube welded to "Swagelok" adapter;* (7) *cap welded to 1½ by 6 in. threaded pipe;* (8,9) *Union type safety head, 1½ in., upper part threaded and welded to 7, lower part welded to reducer, 10;* (10) *reducer;* (11) *4 by 24 in. pipe welded to reducer, 10, and upper flange, 12;* (12) *upper flange;* (13) *1-in. bolts (iron, 8 in all);* (14) *Teflon gasket;* (15) *lower flange welded to 4-in. cap;* (16) *outlet valve and tubing to collection flask;* (17) *aerosol trap with cotton plug at top;* (18) *collection flask with cotton plug;* (19) *Hoke needle valve (brass). Parts can be supplied by Hudson Engineering Co., 1831 28th Avenue South, Birmingham, Ala. The completed apparatus can be supplied by the EE Co., 649 Ponce De Leon, Decatur, Ga.*

pressed between the flanges with eight bolts (13); The tubing and valve (16) at the bottom were for collection of ruptured material. This was collected first in a cotton-stoppered aspirator bottle (17) and then in a cotton-stoppered flask (18). After the material had been collected, the excess pressure was relieved by a valve (19) which exhausted through a coiled tube heated in a muffle furnace. Heated air passed out through a medium sintered glass filter. With these precautions, none of the organisms ruptured was recovered outside of the apparatus or at the filter.

Sterilization was accomplished by closing valve (4) and introducing a suitable germicidal gas. It also could be done by allowing steam to flow from the tube at (16) up through the chamber and out at valve (19). Cooling coils or containers of ice can be placed about the chambers if desired. Fittings and valves were checked under pressure prior to each use for possible leaks.

Rupture of cells. The valve bonnet (19) was removed and the desired volume of cell suspension introduced with the aid of a hypodermic syringe fitted with a 5-in. 20-gauge needle. The bonnet was replaced and valves (19 and 16) were closed. The suspension was saturated with nitrogen gas by gradually increasing pressure to about 1740 psi (or other pressure desired) over a 75-min period. Pressure was then increased until the frangible disc burst and the expelled material collected in the flask (18). By closing valve (16) as soon as the bulk of the liquid was removed, aerosolization was minimized.

Processing of the ruptured material from this point on followed the techniques employed by Foster and Ribi (1960, 1961) for preparing *Brucella* cell walls. Protoplasm was separated from ruptured and unruptured cells by centrifugation at 12,100 × *g* for 15 min at 0 C in a Servall SS-1 head. The sediment was reconstituted in cold water and distributed in small heavy-walled conical centrifuge tubes. It was necessary to treat *B. abortus* sediment with deoxyribonuclease to digest slime in order to separate cell walls from the rest of the sediment. After centrifugation at 3,200 × *g* for 30 min at 0 C in a Servall SP/X head, the tubes contained a clear supernatant fluid and sediment composed of a fluffy upper layer of cell walls and a lower layer of intact cells. The cell-wall layer was dark

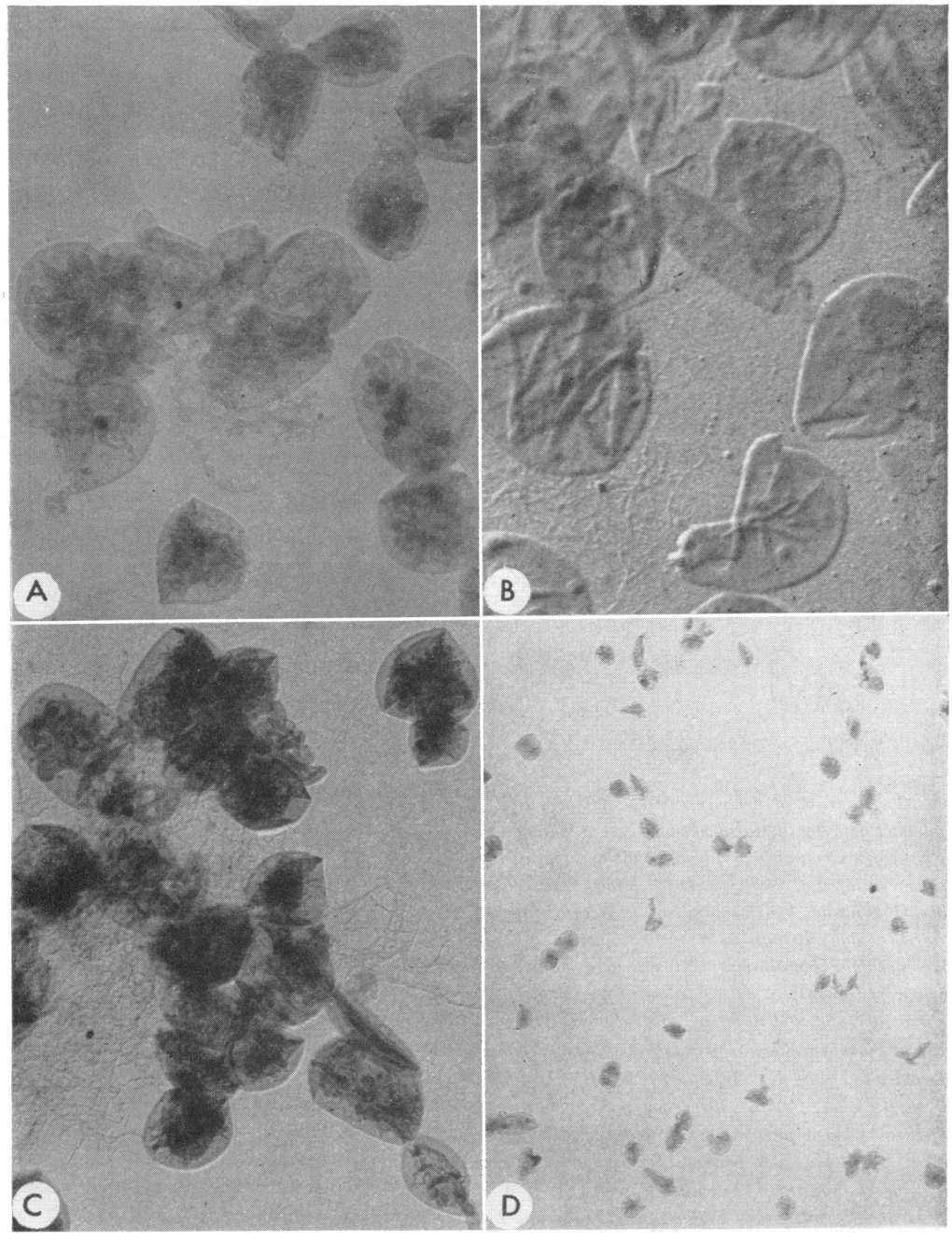

FIG. 2. *Electron micrographs of cell walls produced by explosive decompression.* (A) *Brucella abortus*, (B) *Serratia marcescens*, (C) *Staphylococcus aureus*, (D) *representative field.*

red in preparations of *S. marcescens*. Cell walls were harvested by gently washing off the upper layer with a capillary pipette and water. The cell walls were pooled, resuspended in cold water, recentrifuged at 3,200 × *g* and the upper layer washed off as before and pooled. The pooled suspension was next concentrated by centrifugation at 12,100 × *g* for 15 min at 0 C in a Servall

SS-1 head, resuspended in a minimal quantity of water, and subjected to linear glycerol gradient centrifugation in a 25.1 SW Spinco head at 5,480 × *g* at 5 C for 30 min, using the technique of Ribi and Hoyer (1960). Glycerol was removed by suspending cell walls in water and centrifuging at 12,100 × *g* for 20 min.

RESULTS

After gradient centrifugation, reflected light revealed three visible bands with *S. aureus* and *B. abortus*. Electron microscopy showed that the upper one contained cell walls (Fig. 2), the middle one partially ruptured cells, and the lower one intact cells. *S. marcescens* had four bands (Fig. 3); a top white one which contained miscellaneous small round particles; a second red band which contained cell walls; a third whitish band which contained partially ruptured cells; and a fourth light pink-to-white bottom band which contained intact cells. The red color of the cell-wall bands of *S. marcescens* confirms the finding of Purkayastha and Williams (1960) that the pigment is associated primarily with the cell wall. Protoplasm showed very little pigment. *B. abortus* and *S. marcescens* cell walls were more easily separated from other debris by gradient centrifugation than were those of *S. aureus*.

Concentrations up to 20 mg (dry wt) of cells/ml appeared to be ruptured about as efficiently as lower concentrations. As shown in Table 1, volumes of *S. marcescens* suspensions from 20 to 50 ml yielded preparations which were 31.4 to 58.9% ruptured, and these volume differences demonstrated no consistent effect on rupture efficiency. A single sample containing 125 ml of cell suspension showed 31.9% rupture, which was as good as the poorest efficiency with smaller volumes. All cell suspensions employed in obtaining the data cited were adjusted to equivalent concentrations (62.8 mm³/ml) on the basis of PCV, determined in saline. The cells in water suspensions used for rupture swelled on standing, and the variation in the PCV values of the unruptured cells reflect this. Total solid measurements showed that approximately the same amount of material was in the cell suspension both prior to and after rupture. Unruptured control cells were held in water at the same temperature and for the same time as the ruptured cells.

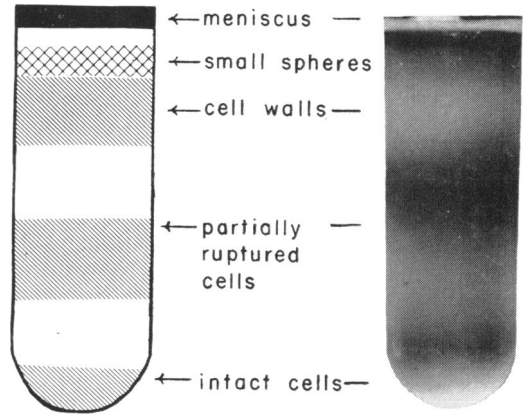

FIG. 3. *Ruptured cell material of Serratia marcescens in a linear glycerol gradient after centrifugation at 5,480 × g for 30 min in a 25.1 SW Spinco head.*

TABLE 1. *Rupture of cells of Serratia marcescens in varied volumes of suspension*

Volume of cell suspension	Unruptured cells*	Ruptured cells*	Ruptured
ml			%
20	114.7	47.1	58.9
20	88.3	40.8	53.7
50	78.5	35.2	55.1
50	83.6	45.9	45.2
50	99.3	55.0	44.6
50	75.4	51.8	31.4
125	72.9	49.6	31.9

* Determined in water. Suspensions had 17.5 to 20 mg/ml (dry wt) total solids prior to rupture and comparable values after rupture. Results are expressed as the packed cell volume (mm³/ml).

S. aureus and *B. abortus* cells (50-ml volumes) with starting PCV of 31.4 to 62.8 mm³/ml, showed rupture efficiencies ranging from 10 to 25% at 1740 psi. *E. tenella* (20-ml volume), with 150 million oocysts per ml, was about 40% ruptured at 1740 psi. The same pressure rather thoroughly broke up *M. gallinarum* and *L. pomona*.

Decompression at 760 psi resulted in much less rupture than at 1740 psi when suspensions of *S. marcescens* and *E. tenella* were tested.

DISCUSSION

Pressures lower than 1740 psi were not very efficient for the rupture of *S. marcescens* and *E.*

tenella. The rupture efficiencies obtained at 1740 psi were somewhat less than anticipated from Fraser's (1951) studies. On the basis of his work, it can be anticipated that increased decompression pressures will result in higher rupture efficiencies. Lower pressures may be indicated for the rupture of certain other organisms, as *M. gallinarum* and *L. pomona* cells appeared to be broken into numerous small fragments by decompression at 1740 psi. Either higher or lower pressures can be readily attained by the use of frangible discs of different ratings.

Another factor which affects rupture efficiency is the degree of saturation of cells with gas. Fraser apparently achieved very efficient saturation by simply shaking the cell suspension in a bottle filled with gas. We found it necessary to allow time for saturation, as the present chamber isn't readily shaken. However, a modification is underway which will permit shaking.

Within the limits of this study, cell concentrations and volumes of cell suspensions have no definite effect on rupture efficiency. It was anticipated that the deeper the column of cell suspension to be ruptured the less rapid decompression would be. This would supposedly result in less efficient rupture. Should this prove to be a problem the obvious solution would be to enlarge the dimensions of the rupture disc and shorten the height of the saturation chamber. Any alteration of dimensions should take into consideration the ratio between the saturation chamber and rupture chamber (1:30 in the present device). A larger ratio should improve rupture efficiency, but more material would be lost on the walls of the rupture chamber.

The decompression chamber and Fraser's system operate at considerably lower pressure than the high-pressure extrusion system employed by Milner, Laurence, and French (1950) and Ribi et al. (1959). The extrusion system probably operates by "tearing" the cells open more than by decompression, for the conditions of operation probably don't allow sufficient time for efficient saturation of the cell suspension with gas. With higher pressures, heat dissipation is more of a problem and much greater mechanical strength is required in the apparatus. There is very little heat generated during saturation in the decompression chamber, and of course, there's an actual heat loss due to gas expansion at the moment of decompression. The modification (Ribi et al., 1959) of the extrusion system is currently more useful for the processing of large volumes of material; but, a decompression chamber could be built to accomodate more material, and possibly heavier concentrations of cells can be used. It is not yet known whether the cell walls prepared from the two types of cells have any differences in immunological or physiological properties.

The cell walls of *S. marcescens* and *B. abortus* were "cleaned up" with little difficulty. There seem to be some spherical particles associated with both, but it is rather difficult to determine whether some of the structures are particles on or in the cell wall or are a consequence of folding over of the wall. Electron-dense bodies were also seen; these were more prominent in *S. aureus* cells, which were not as easy to "clean" as the other cells.

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