Rupture of *Leptospira Pomona* Cells by Explosive Decompression*† (34128)

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(Introduced by Warren C. Eveland)

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Foster *et al.* (1) described an apparatus for the rupture of bacterial cells under conditions of controlled pressure and instantaneous decompression. The method has been used chiefly for the production of eubacterial cell walls, which are retrieved from the cell debris by gradient centrifugation and employed in further studies of their chemical composition and antigenicity (2). While 1740 pounds per square inch (psi) appeared to be the most effective rupture pressure studied for the production of eubacterial cell walls, a strain of *Leptospira pomona* furnished by the present senior author was so completely disintegrated by rupture at this pressure that only tiny fragments of mostly unidentifiable material were detected by electron microscopy (1). The present study was undertaken to determine whether decompression at lower pressures could be used to rupture *L. pomona* cells for the subsequent recovery of salvageable cell structures.

Methods. Preparation of cells. L. pomona strain T-262 was grown for 7 days at 30° in Difco Stuart's medium containing 10% sterile rabbit serum (previously heated at 56° for 30 min) but no added glycerol. The cells from 100–3000-ml amounts of culture were removed by centrifugation, washed once or twice with either 0.85% NaCl or distilled water, and finally resuspended in 40–50 ml of either diluent.

Cell rupture technique. The apparatus used for rupture by explosive decompression has been described by Foster *et al.* (1). The washed cell suspension (40–50 ml) was introduced into the saturation chamber, nitrogen gas was admitted from a pressure tank to give an initial pressure of 300 psi, and a period of 15 min was allowed for saturation of the suspension at this pressure. Additional gas was then admitted to raise the pressure in increments of 200 or 300 psi (with 15 min allowed for saturation after each increment) until the desired rupture pressure was reached. The bottom of the saturation chamber was fitted, in different trials, with different frangible discs designed to rupture at designated pressures: 1740, 1500, 1200, 900, and 500 psi. After rupture of the disc, which releases the cell suspension into the lower "rupture" chamber, excess pressure was relieved through a valve at the top of the saturation chamber, and the suspension of ruptured cells (termed "rupture Ia") was collected through an outlet at the bottom of the rupture chamber. The saturation and rupture chambers were then rinsed with ten 5 ml portions of either cold saline or distilled water, these washings being collected from the outlet, combined, and designated "rupture Ib."

Recovery of cell structures. Two procedures for the isolation of cell structures were attempted. In the first, approximately 10 ml of "rupture Ia" suspension were pressed through a Swinney filter fitted with a

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small Seitz type EK asbestos pad. In the second, "rupture Ia" and "rupture Ib" suspensions were centrifuged separately at 4950 *g* for 60 min. The resulting sediments were combined in 25 ml of distilled water, distributed among eight conical-bottom tubes, and (after addition of 7 ml more water per tube) were recentrifuged at 3440*g* for 30 min. The cloudy supernate and loose, fluffy precipitate forming in these tubes were decanted from the dense sediment in the bottom, combined, and designated "fraction FS." This fraction was subjected to further purification by gradient centrifugation employing discontinuous (0, 10, 20, 30, 40, 50%) and/or continuous (0–50%) sucrose gradients. Gradient preparations were centrifuged at 1100*g* for 2 or 4 hr at 4°C.

Eelctron microscopy. To determine the extent of rupture and to trace certain cell components through the purification processes, various specimens were prepared for electron microscopy. Most specimens were fixed with 1% buffered, isotonic osmic acid (3) using essentially the techniques of Simpson and White (4). Fixed and platinum-palladium (Pt-Pd) or carbon-platinum (C-Pt) shadowed specimens were examined at various magnifications using an RCA-EMU-2E electron microscope.

Results. Grids prepared for electron microscopy before rupture showed typical leptospiral cells exhibiting a protoplasmic cylinder spirally wound about a central axial filament with the whole cell surrounded by an outer envelope. Rupture at 1740 and 1500 psi appeared literally to explode the cells into tiny fragments of unidentifiable material. Intensive scanning of grids prepared after rupture at these pressures revealed only occasional fragments which might be interpreted as tiny pieces of outer envelope or axial filament. On the other hand, grids prepared after decompression from 500 or 900 psi revealed relatively little cell rupture. Final trials were therefore made using 1200 psi. Examination of grids prepared after decompression from 1200 psi revealed mostly fragmented cells—and pieces of axial filament, amorphous protoplasm, and outer envelope could all be detected (Fig. 1).

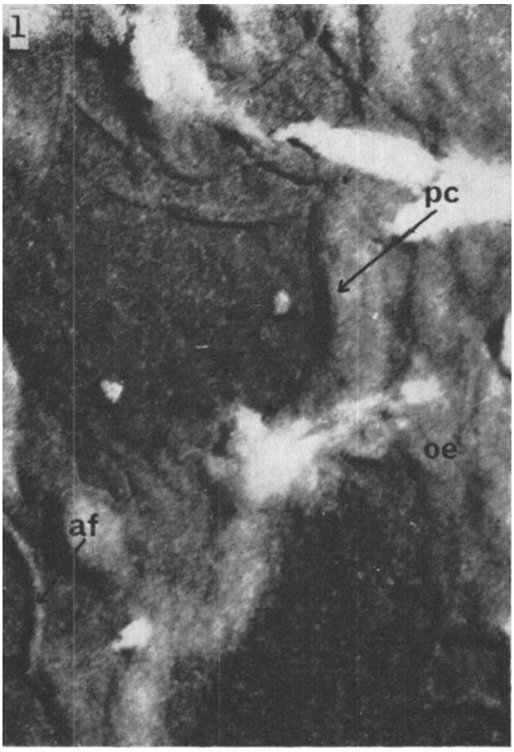

FIG. 1. *Leptospira pomona* cells in rupture Ia preparation obtained by explosive decompression from 1200 psi pressure. Note presence of the three major cell structures: outer envelope (oe), axial filament (af), and protoplasmic cylinder (pc); platinum-palladium shadowed; ×83,000.

Since the three major cell structures appeared fairly well separated, the next logical step seemed to be the isolation of one or more of these cell constituents. Axial filaments appeared to be the structure most likely to be retrieved by fairly simple methods, and as a first attempt, the procedure of filtration was employed. When approximately 10 ml of "rupture Ia" suspension was pressed through a Swinney filter (see *Methods*), grids prepared from material passing through the filter showed nearly pure axial filaments. Segments of varying length were found (Fig. 2), some having terminal knobs and apparently representing the end of attachment while others lacked knobs and apparently were derived from other portions of the filaments. While the filaments appeared almost entirely free of other cell debris (Fig. 2),

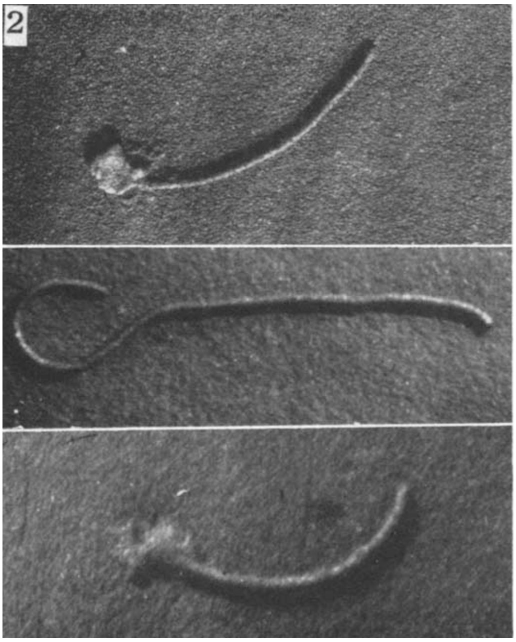

FIG. 2. Axial filaments of *Leptospira pomona* obtained by filtration of rupture Ia preparation through a Seitz filter. Note varying length of segments obtained and absence of any adhering particles of other cell debris; platinum-palladium shadowed; from top to bottom, ×70,000, 78,000, and 90,000, respectively.

the yield of axial filaments was judged to be quite small.

Material from fraction FS (after centrifugation but before gradient separation) was found to contain large quantities of material believed to be outer envelope, but also numerous pieces of axial filament, some intact protoplasmic cylinders, and some intact cells. After centrifugation of material from fraction FS in discontinuous sucrose gradients for 2 hr, a band was found at the 10–20% sucrose interface. Electron microscopic examination of material from this band revealed mostly envelope material, but still some axial filaments and protoplasmic cylinders could be observed. In a final attempt at further purification, material from these bands was collected, centrifuged at 41,000*g* for 4 hr, washed once with distilled water, and placed on continuous (0–50%) sucrose gradients. These preparations were centrifuged for 4 rather than 2 hr in an attempt to separate outer envelope from the presumably heavier protoplasmic cylinders and axial filaments. A more diffuse band, ranging to the 30% sucrose region was obtained, and samples removed from the uppermost part of this band were found to consist mostly of the presumed outer envelope; however, some pieces of axial filament were still present (Fig. 3).

Discussion. Most investigations of the chemical composition and antigenic properties of leptospiral cell components have employed chemical treatments to free various cell structures or release antigenic fractions. A more valid approach would seem to be the separation of cell structures or fractions by purely physical means less likely to alter the technical properties or antigenicity of the components under investigation. It is believed that the work reported here, as well as a recent report by Nauman (5), represent initial steps in this direction. Nauman isolated axial filaments of "water leptospirae" in good quantities from sonically disrupted

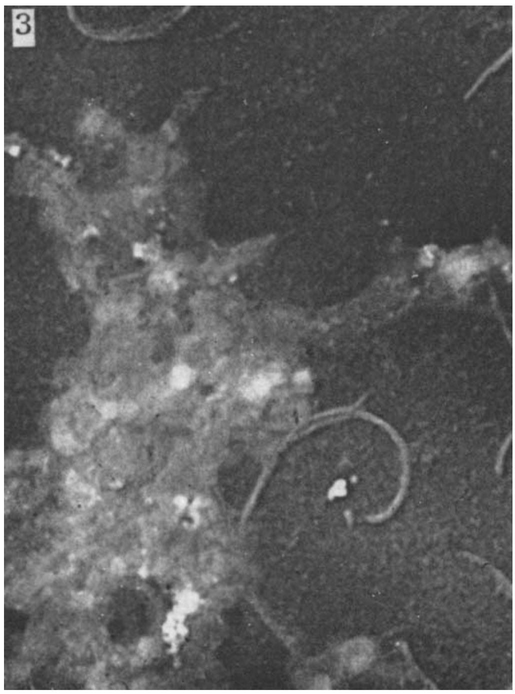

FIG. 3. Cell constituents of *Leptospira pomona* after sucrose gradient centrifugation of fraction FS. Some axial filaments still remained in this preparation; carbon-platinum shadowed; ×80,000.

cells by differential, isopicnic, and zonal centrifugations. In some cases, however, he had used sodium desoxycholate for cellular disruption.

In the present study, axial filaments of *L. pomona* were isolated by strictly physical means in high purity, but the quantities obtained were rather small. Presently, a filter assembly is being adapted to fit the outlet of a modified decompression apparatus in attempts to employ large scale filtration of ruptured cell suspensions for the production of axial filament material in quantities adequate for chemical and serological study.

Attempts at isolating outer envelope material were less successful in that only partial purification was achieved, some axial filaments remaining in the final product. The main objective of this investigation, however, was to establish that purely physical means could be used to disrupt leptospiral cells with release of salvageable cell structures.

Summary. Cells of *Leptospira pomona* were successfully ruptured by an explosive decompression technique. Twelve hundred pounds per square inch appeared to be the most favorable pressure for rupture with subsequent recovery of salvageable cell parts. Electron microscopic observations revealed that axial filaments could be recovered from ruptured cell suspensions by filtration—in high purity but rather low yield. Material believed to be derived from the outer cell envelope was similarly shown to be considerably purified by differential and gradient centrifugation; however, some axial filament fragments remained in these preparations. The method of explosive decompression is suggested as a physical means for the isolation of leptospiral cell structures for studies of chemical composition or antigenicity.

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