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Use of Pressure Cell to Prepare Cell Walls from Mycobacteria.* (24730)

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In a recent report(1) separation of cell wall from internal protoplasm of mycobacteria based on the principle used by Salton and Horne(2) was described. Although yields of cell walls obtained were small, they permitted limited biologic analyses which demonstrated the immunologic significance of this fraction. The demand for larger quantities of cell wall material was met by utilizing a commercial pressure cell in place of the Mickle apparatus (1,2) to achieve selective disruption of organisms. In the resulting cell wall fractions, however, considerable amounts of denatured protoplasmic substances adhered to inner surface of walls and could not be washed off. Further investigation revealed that an increase in temperature at the orifice of the needle valve during bleeding of very highly compressed bacterial suspension apparently caused partial denaturation of protoplasmic components. Since conventional cooling systems were not adequate, an experimental instrument was devised which allowed sufficient cooling of the orifice and needle by exposing them to compressed CO_2 released at a separate needle valve. This paper describes application of such a pressure cell for preparation of cell walls of mycobacteria.

Description and operation of pressure cell. The instrument (Fig. 1) consists essentially of a steel cylinder and piston (A) and 2 needle valve systems (B and C). The outlet at bottom of cylinder is connected by steel tubing to the body of the high pressure needle valve (B) used for bleeding the compressed bacterial suspension. This connection included a high pressure stainless steel nipple, gland nuts, and compression sleeves purchased from American Instrument Co., Silver Spring, Md. The 3-way cross-type stainless steel valve (B) with compression packing was ordered from the same company. Its upper opening (D) is connected by steel tubing to the expansion chamber of the needle valve C (brass body and steel needle) used to bleed compressed CO_2 to cool the needle and orifice of valve B. The lower opening (E) is connected by plastic tubing with suction flask from which glass tubing leads to closed hood equipped with exhaust fan and incinerator. The piston ring, a Parker O Ring No. 2-218, made of compound 49-031, was obtained from Parker Seal Co., Clevland, O. *Operation.* Piston and cylinder were cooled in ice water and the cylinder was then filled with the cold mycobacterial suspension (maximum quantity about 100 ml).

* We are indebted to Montana Trudeau Society for part of equipment needed.

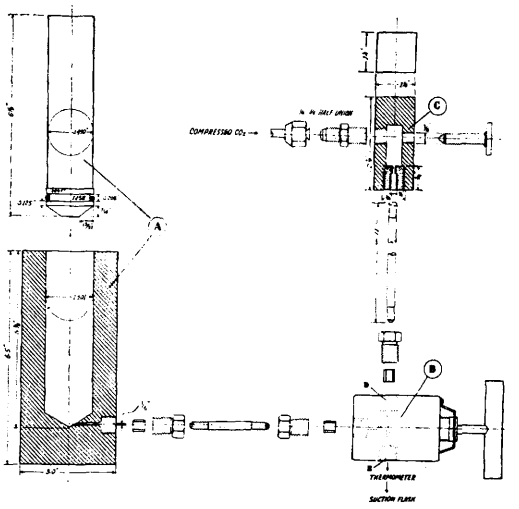

FIG. 1. Diagram of pressure cell used for preparation of mycobacterial cell walls.

Needle valve B was cooled by bleeding CO_2 with valve C until temperature of CO_2 stream, measured near opening E, was maintained at about 2°C. The bacterial suspension was then bled at valve B while a total load of 35,000 to 40,000 lb was maintained on the piston using hand-operated hydraulic press. Valve B was adjusted so that temperature of bacterial products leaving the orifice did not rise above 15°C. Higher temperatures could be avoided either by reducing the flow of the suspension with valve B or by increasing the CO_2 stream with valve C. The suspension was collected in the suction flask which had been placed in ice water bath. The CO_2 vapor, together with aerosol of bacterial material, escaped through the glass tubing to the incinerator.

Preparation of cell walls. *Mycobacterium butyricum* and 2 strains of *M. tuberculosis*, BCG and H37Ra, were used. The organisms were grown, harvested, and washed as described previously (1). Washed bacteria were dispersed in 0.5% solution of Tween 80 to a concentration of about 3 mg/ml and processed in the pressure cell as described above. All of the following procedures were performed at 4°C the same day the cells were harvested. The mixture resulting from disruption of cells by rapid change of pressure and velocity at orifice was centrifuged at 5000 g for 20 minutes. The supernatant contained dispersed protoplasm, and the sediment consisted of cell walls and unbroken cells. The sediment from each 100-ml portion of starting suspension was resuspended in enough Tween solution to fill three 12-ml conical tubes and centrifuged at 2000 g for one hour. These sediments consisted of a loose, translucent upper layer of cell walls and a hard packed, opaque lower layer of unbroken cells. The cell walls were washed off with Tween solution and further purified by centrifugation and resuspension in distilled water. Electron micrographs showed that the resulting cell walls contained little, if any, protoplasm. Also, whereas cell walls from the Mickle apparatus were usually ruptured at one end of the rod, part of the walls from the pressure apparatus were split perpendicular to the long axes of the cells into a small number of units (Fig. 2). Difficulties encountered during processing disruption products of the Mickle apparatus were not experienced, and nondispersible aggregates were not formed when the pressure cell was used. Therefore, yields of cell walls from a given amount of starting material were much larger. On a dry weight basis, about 60 mg of purified cell walls were obtained per gram of intact cells of the starting material.

Summary. Use of a pressure cell for preparation of purified cell walls of mycobacteria is described. This technic eliminated difficulties encountered earlier with the Mickle pro-

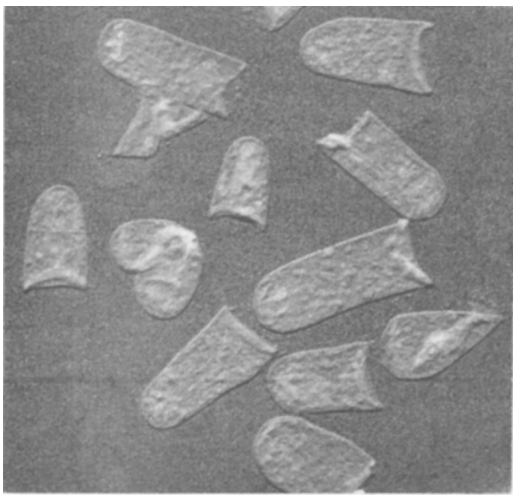

FIG. 2. Cell walls of *Mycobacterium tuberculosis* (BCG). Magnification 64,000 ×.

cedure and the yields were considerably increased.

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Biologic Reactivity of Antigen and Antibody in Specific Precipitate.* (24731)

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Study of effects of union of antigen with antibody upon specific functional properties of either is of significance in understanding the immune process. Recently, it has been reported that soluble complexes of antigen and antibody can cause immediate reactions which resemble systemic or local anaphylaxis (1,2). Our report(3) indicated that antigen-antibody complexes injected as mixtures in equivalence or in great antigen excess could sensitize local skin sites in guinea pigs so that subsequent challenge by intravenous injection of antigen specifically elicited local anaphylactic reactions. The present study indicates ability of washed specific precipitate, injected intravenously, to sensitize to systemic anaphylaxis when injection of antigen is given subsequently. Furthermore, washed specific precipitate, injected intravenously, acts like antigen to elicit local anaphylactic reactions at skin sites previously sensitized with antibody alone. Thus, the specific biologic reactivity of both antigen and antibody is demonstrated in the complex formed by their union.

Materials. The antigen used was 4 times recrystallized hen egg albumin. Rabbit and guinea pig antisera were analyzed for antibody nitrogen by quantitative precipitin studies(4). Specific precipitate was prepared at equivalence or slight antigen excess as determined by appropriate supernate tests. The precipitate was washed 3 times in cold saline similar to the technic used for preparation of specific precipitate for quantitative estimation of antibody. Washed specific precipitate containing known amounts of antibody and antigen was then suspended with vigorous agitation in chilled saline to make suspensions containing 15 to 140 μg AbN/ml. There was evident variation in particle size of these suspensions. Different preparations of specific precipitate varied somewhat in sensitizing capacity. It has not been determined whether these differences are due to variations between differing antisera or to small differences in ratio of antigen to antibody in the precipitates. The data are presented for ranges of concentration of antibody. The guinea pigs used for systemic or cutaneous anaphylaxis were albino males weighing 250 $\pm$ 50 g.

Results. Systemic anaphylaxis. To determine if specific precipitate could sensitize to subsequent challenge injection of antigen, the following experiment was conducted. Guinea pigs were injected intravenously with washed specific precipitate containing from 15 to 140 μg AbN (rabbit). The recipients of these amounts of specific precipitate tolerated the injections well, although with larger amounts of specific precipitate, signs of mild anaphylactic reaction were occasionally observed. At varying intervals thereafter, 1 mg egg albumin was injected intravenously and the animals observed for manifestations of systemic anaphylaxis. Reactions were graded as follows:

0 No reaction; + Chewing, nose scratching, bristling of hair; ++ Coughing, pronouced respiratory embarrassment; +++ Loss of stability, convulsions; ++++ Death. About half of the animals which survived the respiratory component of anaphylaxis showed additional signs of prostration in varying de-

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