Production Model Press for the Preparation of Bacterial Cell Walls

T. D. Perrine, E. Ribi, W. Maki, B. Miller, and E. Oertli

National Institute of Arthritis and Metabolic Diseases and the National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

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Abstract

Perrine, T. D. (National Institutes of Health, Bethesda, Md.), E. Ribi, W. Maki, B. Miller, and E. Oertli. Production model press for the preparation of bacterial cell walls. Appl. Microbiol. **10**:93–98. 1962.—A modification of the apparatus previously described permits the preparation of cell walls in quantity. This consists of a heavy duty, double-acting hydraulic press with motor-driven pump, and a superstrength alloy steel pressure cell which is corrosion resistant. Liquid cooling of the jet is substituted for the previously used gas cooling to minimize aerosol formation and to facilitate subsequent treatment of the products. The device produces cell walls of excellent quality in good yield. The pressure cell has been used satisfactorily up to about 60,000 psi. Design details are given.

Recently, we have described an apparatus (Ribi et al., 1959) which could be used to prepare cell walls of mycobacteria. This consisted of a pressure cell (in some respects similar to the French type) and single-acting hydraulic press which forced a single charge of a bacterial suspension through a specially cooled needle valve orifice at very high velocity. Experiments with other bacteria proved that this apparatus could be used for the preparation of cell walls of many other species (Ribi, *unpublished data*), and because of our requirement for these materials in great quantity, we designed a production model apparatus suitable for large batch or continuous flow operations. This device will process 1 liter of bacterial suspension in 7 min at 35,000 psi. It has a rated capacity of 60,000 psi, and has occasionally been operated at 71,000 psi. (These calculated pressures neglect frictional losses in the rams.)

The original device referred to above employed a stream of cold gas to dissipate the heat generated at the orifice of the pressure cell. We have found this to be the method of choice for small-scale (1 or 2 gal) batch operation. However, the press described in this paper is capable of processing 70 ml of suspension in 11 sec at 35,000 psi. This is equivalent to the generation of 372 cal of heat per sec. Allowing an 8 C temperature rise for the process fluid, 321 cal per sec are still left to be dissipated. Roughly, 20 to 40 ft^3 per min of N_2 at −80 C would be required, even assuming 100% efficient heat exchange. This emphasizes the obvious disadvantage of gaseous cooling, namely the creation of a voluminous bacterial aerosol. Other disadvantages of a gaseous coolant as compared to the use of a liquid are much lower heat capacity in calories per sec at the jet, the danger of freezing at the jet, and the impossibility of further immediate chemical (or physical) treatment of the press effluent with enzymes, solvents, and the like, which may prove desirable for certain preparations. It should be emphasized, however, that gas cooling is inherently cleaner, and its use greatly simplifies the operation for small runs. The use of the gas-cooling principle is satisfactory when the stroke time is increased from 11 sec to 5 min, whereby a N_2 stream of only 20 to 40 ft^3 per hr suffices to cool the orifice. The rate of aerosol generation is then reduced to the point where the effluent may be collected in a closed container which is connected to an incinerator equipped with a fan, thus greatly reducing the hazard.

The instant ejection of the effluent from the jet into a large, high velocity stream of ice-cold liquid gives the most rapid cooling possible. It also results in a large dilution of the products and, although most of these may be recovered by centrifugation, the volumes of solution to be handled are considerably increased. However, we regard the aerosol problem as so serious a health hazard that we have made use of liquid cooling in this factory-scale press. For small-scale operation, where clean-up time is an important factor, or where a large dilution of the product is to be avoided, we have found it a simple matter to adapt the apparatus described below to gaseous cooling by replacing valve *8* (Fig. 1) with the valve described in our earlier paper (Ribi et al., 1959).

The entire apparatus is shown in Fig. 1 and 2, whereas Fig. 3 is a schematic representation of one alternative path of the bacterial suspension through the system. The pressure cell is shown in Fig. 4, 5, and 6, and the essential details of the hydraulic press are represented in Fig. 7. These drawings give the important dimensions.

The large-scale cooling requirement was satisfied by collecting the pressed suspension immediately as it passed through the orifice in a "pickup liquid" supplied at 1 C at a rate of 3½ liters per min. The "pickup liquid" can be altered to meet the requirements of the process, but is ordinarily 0.5% Tween 80.[1] We envisage that various organic liquids, enzyme solutions, buffers, and the like,

[1] Polyoxyethylene sorbitan monooleate; Atlas Powder Company, Wilmington, Del.

may eventually prove useful for this purpose. Using 0.5% Tween 80, the temperature of the effluent from the needle valve does not ordinarily exceed 7 C. The liquid is circulated through a heat exchanger which consists of a coil of stainless steel tubing immersed in brine. A Vanton XB-S 12 pump,[2] which we fitted with a cooling jacket, is used to circulate the fluid. Tubing is ¼ in. inside diameter (i.d.) stainless steel.

An expansion reservoir is provided to accomodate the combined volume of the pickup liquid and the pressed suspension. These combined liquids are continuously recirculated, but as the volume increases, and the content of bacterial products rises, some of the fluid is bled off into a storage bottle (not shown in Fig. 1). Fresh pickup liquid can be added to control the composition of the circulating fluid. With the use of this system, a certain amount of dirt contamination of the product has been encountered.

The feed for the pressure cell is contained in a reservoir equipped with a cooling coil, all containers for the processed liquid being likewise cooled, as is the pressure cell itself. We have made provision to feed the processed bacterial suspension directly into a Sharples[3] centrifuge for separation of the suspended products. The entire device, including the centrifuge, is enclosed in a sealed chamber which is maintained under negative pressure. The exhaust air is incinerated. The interior parts of the apparatus may be sterilized with live steam and with disinfectant solution.

[2] Vanton Pump and Equipment Company, Hillside, N. J.

[3] The Sharples Corporation, Philadelphia, Pa.

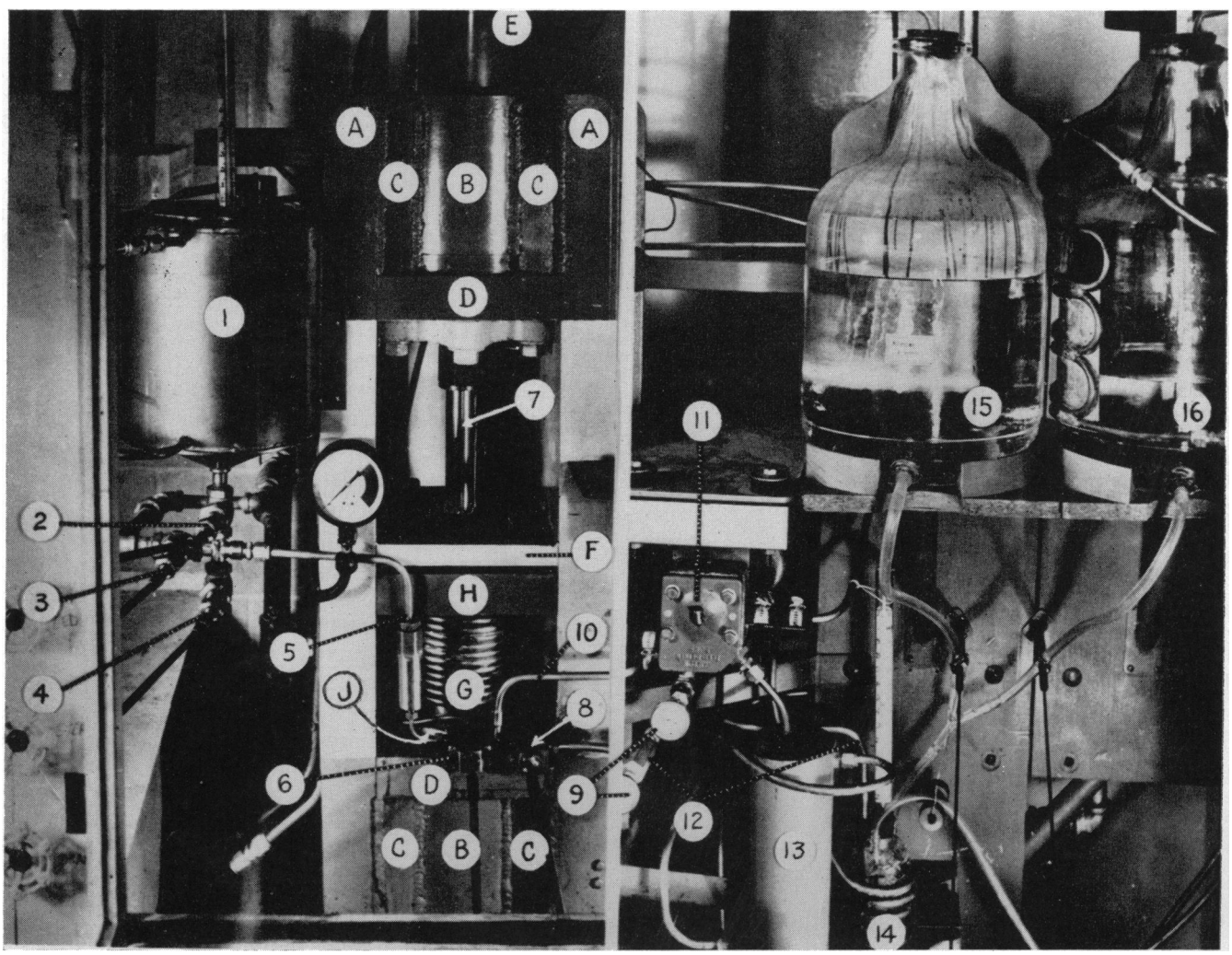

FIG. 1. *Cell wall press with liquid cooling system. The storage reservoir and the centrifuge are not shown.*

(1) Feed reservoir, (2) feed control valve, (3) steam inlet valve, (4) drain valve, (5) feed delivery tube and check valve, (6) feed needle valve, (7) piston of pressure cell, (8) pressure-cell orifice, (9) thermometers for pickup fluid inlet and exit tube, (10) pickup fluid inlet tube, (11) pump for circulating pickup fluid, (12) pickup fluid exit tube, (13) pickup fluid heat exchanger, (14) expansion reservoir, (15) disinfectant storage bottle, (16) pickup fluid storage bottle.

(A) Press side member channels, (B) 8 in. pipe, (C) ½ in. plate reinforcements, (D, D') press platens, (E) hydraulic ram, (F) intermediate plate, (G) pressure cell, (H) plate holding pressure cell, (J) guide ring.

The Pressure Cell[4]

Several types of pressure cells were tried before a satisfactory design was developed. Attempts to use the cell previously described led to early O-ring failure and to permanent deformation of the cylinder and piston.

When the hydraulic pressure in a cylinder exceeds the elastic limit of the steel used, plastic flow occurs, and the usual formulas used for the design of hydraulic cylinders no longer apply. Increasing the wall thickness of such a cylinder may delay failure, but will not prevent it. When we subjected cylinders (made of 303 and 316 stainless steel 3 in. outside diameter (o.d.) by 1.5 in. i.d.) to 70,000 psi, the bore was permanently enlarged about 0.030 in. Similar results were obtained with a carbon steel cylinder. These deformed cylinders have increased resistance to further deformation because of the prestressed condition in the outer layers of steel ("autofrettage," or self-hooping principle). They can be cautiously rebored to true cylinders which show greater resistance to deformation than initially, within the limits of the deforming stress, but this effect does not operate when the pressures used approximate the elastic limit of the steel. Since it was likely that our work would require pressures nearly as high as these deforming pressures, we decided to use a stronger cylinder. There are available certain superstrength alloy steels, such as AM350 and AM355,[5] which can be used for this construction. These have the advantage of showing high resistance to corrosion by aqueous solutions. We therefore obtained a forged billet of AM355 steel, 5¼ in. in diameter, which was bored initially to 1 7/16 in. and heat treated. A temporary piston was made, the cylinder filled with glycerine, and hydraulic pressure generated to 75,000 psi. Three such expansions were used in an attempt to strengthen the steel by the autofrettage method, but no permanent expansion of the bore was obtained. The cylinder was then carefully machined and given a high polish to 1.491 in. An excellent finish is essential to prevent

[4] A pressure cell and orifice based on this design are being manufactured by the American Instrument Company, Silver Spring, Md.

[5] Allegheny Ludlum Steel Corporation, Pittsburgh, Pa.

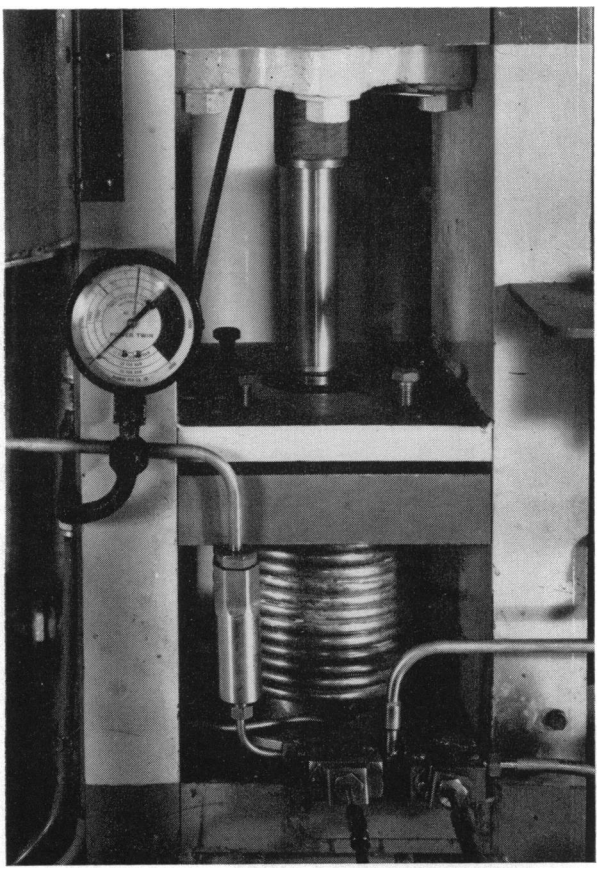

FIG. 2. *Pressure cell mounted in the hydraulic press, showing the valve arrangement and cooling coil.*

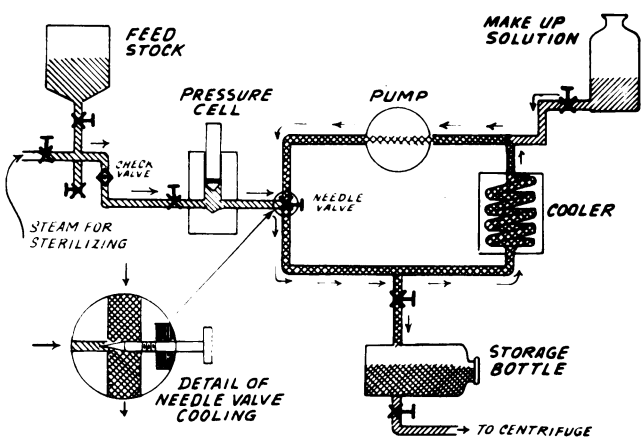

FIG. 3. *Schematic flow diagram for liquid cooling*

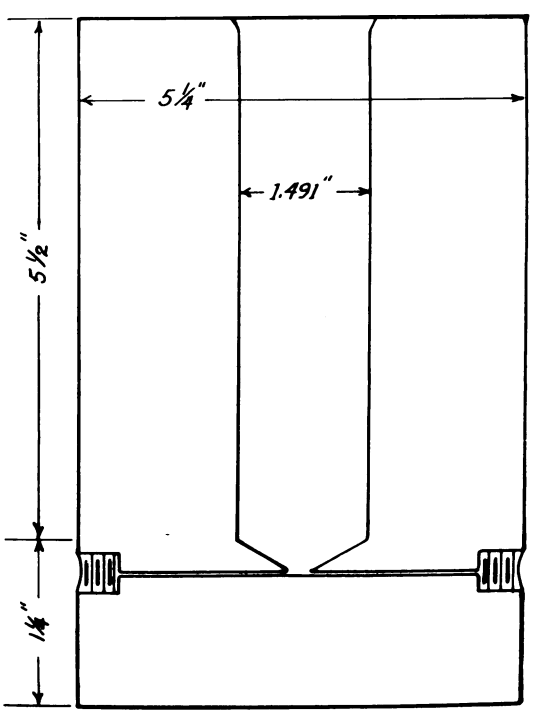

FIG. 4. *Pressure cell cylinder*

O-ring damage. The heat treatment was done according to the directions of the supplier[5] and consisted of holding at 1,750 F for 1 hr, followed by a water quench, then −100 F for 3 hr, and retempering at 850 F for 3 hr. The finished piece had a Rockwell C (R_C) hardness of 48, and was just machinable. The 0.2% yield strength would be expected to be about 195,000 psi, the tensile strength 223,000 psi, and the elongation 10%. The safety factor for our application is probably at least 2.

The pressure cell was provided with two outlets at the bottom of the bore, to receive ¼-in. "Aminco"[6] superpressure tubing, as illustrated in Fig. 4.

In high pressure work of this sort, the piston must fit the cylinder very closely, or the rubber O-ring will be extruded. Since this action is never symmetrical (i.e., the O-ring ruptures on one side only), the piston is subjected to a very strong lateral force which may result in scoring the cylinder wall and piston opposite the point of O-ring failure.

Both the piston and O-ring expand under load, and we consider attempting to match the expansion of one to the other. Unfortunately, at these high pressures, the rates of expansion are not apt to be linear. Moreover, in our design, in which the cylinder is rather short and has an integral bottom, and where the O-ring is on the piston, the strain in the cylinder varies with the stroke, whereas the expansion of the piston is substantially uniform. This problem can be overcome by using a soft metal backup ring on the piston above the rubber O-ring. The soft ring is expanded by hydraulic pressure to fit tightly against the cylinder wall. When the pressure is released, the cylinder squeezes the soft ring back nearly to normal size, but leaves a little residual tension against the cylinder wall. This principle was applied successfully to the design of a new piston (Fig. 5 and 7).

[6] American Instrument Company, Silver Spring, Md.

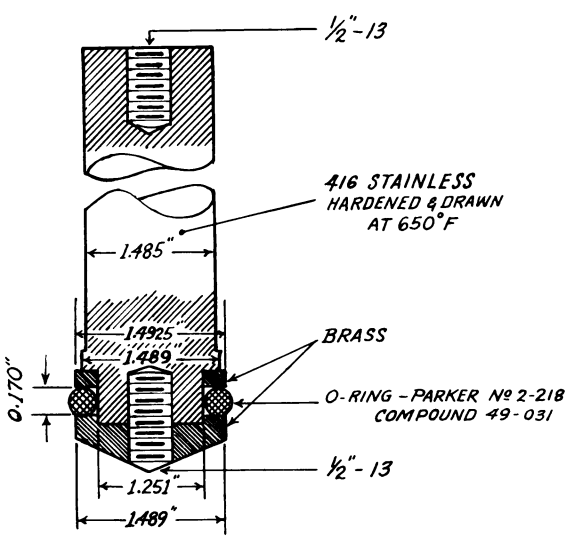

FIG. 5. *Pressure cell piston*

This piston was constructed of 416 stainless steel, hardened, and drawn at 650 F to give an R_C hardness of 37. It should be noted that we have inverted the usual procedure of using a very hard piston in a softer cylinder. This was done to protect the more expensive cylinder. Dimensions and details of construction are shown in Fig. 5 and 6. The backup ring and nose cone were made of brass, but could advantageously be made of a material which is physiologically more acceptable, since a slight contamination of the bacterial products with the metal is observed. The Parker no. 2-218, compound 49-031, O-rings previously specified were found to be eminently satisfactory.[7]

In operation it was found that this pressure cell gave greatly improved performance over the previous model. The behavior of the brass backup ring leads us to believe that there is no serious expansion in the cylinder bore under load. The maximal expansion on the outside diameter of the cell is negligible at 70,000 psi. The O-ring shows substantially no damage under these conditions, a single ring sufficing for several presses at 70,000 psi, or for many liters of solution at 45,000 psi.

[7] Parker Appliance Company, Cleveland, Ohio.

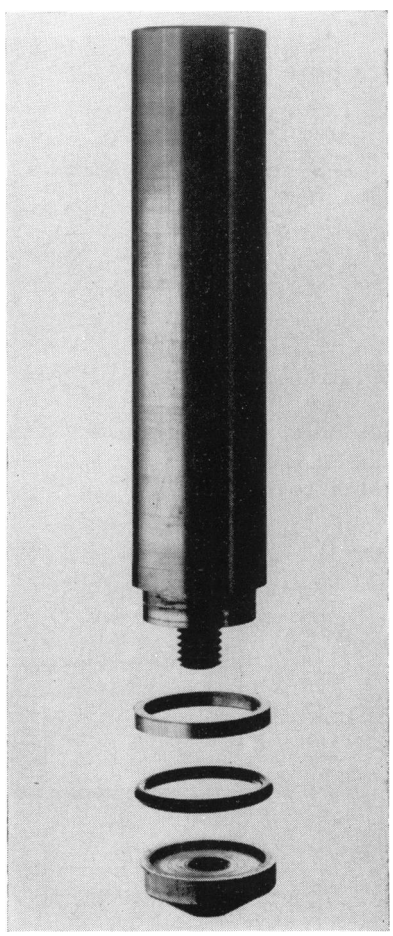

FIG. 6. *Assembly of pressure cell piston packing*

The Pressure-Cell Orifice[4]

An Aminco no. 44-6145 needle valve[6] was used. This is a T-type needle valve designed for 60,000 psi operation, with the needle closing the stem of the T. The passage forming the top of the T was bored out ¼ in. to permit more facile passage of the cooling fluid (Fig. 3). It was necessary to remodel the packing gland to permit this modification. The controlled passage of the valve was connected to the pressure cell, the valve then being connected into the pump circuit by means of the other two openings. In this way, the pressed liquid, which is heated by friction as it passes through the jet, was at once picked up by the cooling liquid, which also serves to cool the valve body.

The Hydraulic Press

The development of pressures of the order of 60,000 psi in this pressure cell requires a load of 100,000 lb. Because of the tight fit of the piston in the cylinder of the pressure cell, and also to facilitate production operation, a double-acting hydraulic ram was required which would drive the piston of the pressure cell either up or down. We constructed a press frame to hold this ram and the pressure cell in such a way that deflection of the axis of the press under load would be held to a minimum. This was achieved by making the tension rails of the press of ample size, and by keeping the platens narrow, and of ample and symmetrical design. The general layout is illustrated in Fig. 7.

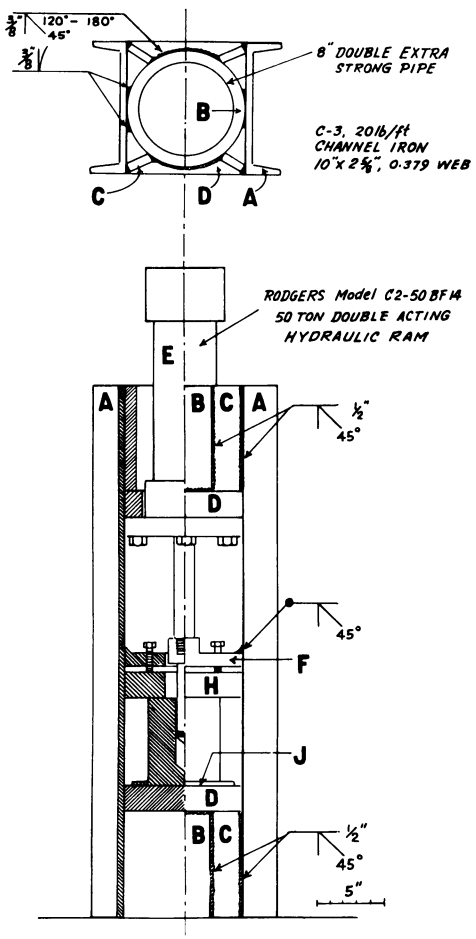

FIG. 7. *Hydraulic press. (See text for dimensions)*

The press is of welded construction. The side members are two C-3 20 lb per ft channel sections (*A*) each 49½ in. long. At each end of these side members, an 8-in. length of 8-in double, extrastrong steel pipe (*B*) is fillet welded to the webs of the channels, with the axis of the pipe coincident with the axis of the press. In addition to these weldments, four 8-in. strips of ½ in. plate (*C*) are fillet welded to the pipe and to the edges of the channel. Thus, each half of the pipe is supported by four 8-in. fillet welds, which provide a combined area in shear of nearly two and one-half times the cross-sectioned area of the channel.

Each platen of the press consists of a 2-in. steel plate (*D*) and *D'*) welded in position against the thrust-bearing surfaces of the pipes. The upper plate (*D*) is machined to receive the hydraulic ram cylinder (*E*), which is a Rodgers 50-ton double-acting unit[8] with 14-in. stroke, model C-2 50BF14. This is bolted to *D*. Subsequently, intermediate plate (*F*) is welded in place. This plate, 1-in. thick, is machined with a center hole 3-in. in diameter, and drilled and tapped for four 1/2-13 thrust screws on a 7-in. diameter bolt circle.

The pressure cell (*G*) is held in place by plate *H* (which has a 3-in. hole machined in the center), and is, in turn, held in position by the thrust screws in plate *F*. To aid in centering the cell in the press, a guide ring (*J*) is screwed to plate *D'* after the exact axis of the press has been determined by experiment. Arrangement is made to bolt the press to a suitable worktable.

It is necessary to proceed cautiously when welding the components of the press together so that warping and misalignment may be avoided. However, the symmetry of the structure minimizes this problem, and we were able to maintain *D'* perpendicular to the axis of the press within one- or two-thousandths of an inch. The axis of the press does not appear to shift under load, and there is no evidence of localized stress or permanent strain at the maximal tested load of 125,000 lb.

The hydraulic ram is driven by an Owatonna Tool Company no. G250-1 hydraulic pump,[9] which has a capacity of 10,000 psi, and is equipped with a throttling-type reversing valve, which permits perfect control of the ram speed, force, and direction.

Cell Wall Production

As stated above, the choice of gas or liquid cooling for cell wall production depends very largely upon the rate at which it is desired to process the bacterial suspensions, and the importance of avoiding aerosol formation. Since, at the

[8] Rodgers Hydraulic, Inc., Minneapolis, Minn.

[9] Owatonna Tool Company, Owatonna, Minn.

time of this writing, we have not undertaken large-scale production of cell walls, most work has been done with the gas-cooling system described previously, and adapted to the present hydraulic system and press. Care is exercised to confine the aerosol.

This adaptation is straightforward, and requires no separate description. The results are being reported separately and are similar to those previously reported (Ribi et al., 1959).

Limited trials of the liquid-cooling system have been made, using the electron microscope to evaluate the quality and yield of cell walls produced by this process. These trials have uniformly produced cell walls of excellent quality in good yield.

In addition, using standard culture techniques, a study has been made of the dissemination into the surrounding atmosphere of viable *Serratia marcescens* during the preparation of its cell walls by the liquid-cooling method. Very few colonies of this organism were obtained within the apparatus housing until the centrifugation step. Since an open-type Sharples supercentrifuge was employed, it is not surprising that very heavy contamination was encountered in the latter operation. We assume, although we have not demonstrated it, that contamination by disrupted bacterial particles is also limited very largely to the centrifugation step.

ACKNOWLEDGMENTS

The pressure cell was constructed by the Instrument Section of the National Institutes of Health. The heat treatment was done by the National Bureau of Standards, Washington, D. C. We are greatly indebted to John Foster, School of Veterinary Medicine, Athens, Ga., for the culture tests with *Serratia marcescens*.

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RIBI, E., T. PERRINE, R. LIST, R. BROWN, AND D. GOODE. 1959. Use of a pressure cell to prepare cell walls from mycobacteria. Proc. Soc. Exptl. Biol. Med. **100**:647–649.