times in distilled water and treated with 1% OsO_4 for 5 min. Dehydration and embedding were according to the procedure described previously by Voelz and Dworkin. The photographs in Fig. 1 to 3 are sections through cells representative of the cell populations of each of the species.

Adenosine triphosphatase activity was present in all three species investigated, as demonstrated by lead deposits in the cell. These deposits presumably result from the enzymatic cleavage of ATP and the subsequent precipitation of orthophosphate by $Pb(NO_3)_2$, one of the components of the medium. In controls minus ATP, none of the three species tested had lead phosphate deposits. The adenosine triphosphatase reaction is specific, in that orthophosphate is not liberated from a wide spectrum of organic phosphorus compounds, including adenosine monophosphate (*see* Hughes). Electron micrographs demonstrate that the sites of adenosine triphosphatase activity vary among the species tested. In *E. coli* (Fig. 1) adenosine triphosphatase activity was found predominantly in the cytoplasmic membrane and cell wall. In *B. cereus* the enzyme activity was located in the cytoplasmic membrane, cytoplasm, and the nuclear region (Fig. 2). Enzyme activity was also present around the vacuole. No activity was found in the cell wall of *B. cereus*. Adenosine triphosphatase activity in *M. xanthus* (Fig. 3) was found only in the cytoplasm. It is uncertain that the three species were harvested and fixed at the same stage of their particular growth cycle. Enzyme activity or the supply of ATP can possibly vary in certain areas within the cell at different stages of growth. Since adenosine triphosphatase has been isolated from contractile proteins of dividing higher cells, and since a change has been observed in the site of adenosine triphosphatase activity in bone-marrow cells of mice, depending on the stage of differentiation or mitotic cycle (Voelz, *unpublished data*), it is conceivable that adenosine triphosphatase can occur in the cytoplasm of bacterial cells, in the cell wall, or in the cytoplasmic membrane, depending upon their stage of development. This could explain the diversity of adenosine triphosphatase sites found in different bacterial species. Further studies will be necessary to determine whether physiological conditions during growth and morphogenesis influence the sites or activity of this enzyme.

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IMPROVED TECHNIQUE FOR THE PREPARATION OF STREPTOCOCCAL CELL WALLS

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An efficient means for the mechanical disruption of bacterial cells in a Mickle disintegrator with subsequent separation of the cell walls from the cytoplasmic contents by differential centrifugation was introduced by Salton and Horne (Biochim. Biophys. Acta **7**:177, 1951). In this procedure, a suspension of bacteria is vigorously shaken with glass beads; although excellent cell-wall preparations devoid of cellular material can be achieved, considerable time is required to process large batches of bacteria. For this reason other disruption equipment has been employed for batch work, such as the Waring Blendor and the Virtis homogenizer. One of the major disadvantages of these instruments is that the glass beads abrade the metal blades, releasing fine metal particles which cannot be separated from the cell walls with subsequent centrifugation. This technical report describes the disintegration of hemolytic streptococci with a Braun homogenizer (Bronwill Scientific, Rochester, N.Y.). The use of this instrument has proven particularly advantageous, because large batches of streptococci can be efficiently and effectively disrupted in a short time and the isolated cell walls are devoid of metallic particles. The data indicate

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that the cell walls obtained with this homogenizer are comparable to those obtained with the use of the Mickle disintegrator.

The homogenizer unit consists of a one-half horsepower motor and an oscillating chamber assembly which houses the inner flask chamber. The material to be homogenized is placed in a 75-ml Duran flask, together with glass beads. The flask is inserted into the inner flask chamber which is connected to an eccentric cam. The resulting elliptical shaking motion effectively mixes and rolls the material. The belt and pulley system can be adjusted to shake the chamber at 2,000 or 4,000 oscillations per min. A stream of CO_2 delivered to the inner chamber by a flexible capillary tube is an effective means to cool the material.

Group A-variant streptococci (strain K43 variant) were grown in 20 liters of Todd-Hewitt broth, harvested, and washed three times with distilled water. The wet weight of the packed cells was 45 g. The collected cells were diluted 1:4 (v/v) with distilled water. To a 75-ml Duran flask were added 30 g of size 12 glass beads (0.17 to 0.18 mm; Bronwill Scientific), 30 ml of the bacterial suspension, and 0.2 ml of tri-*n*-butyl phosphate. The stoppered flask was shaken for 3 min at 4,000 oscillations per min with sufficient CO_2 delivered to the chamber to prevent heating. Essentially all of the bacteria were disrupted in 3 min. Examination of a Gram-stained preparation with a light microscope revealed homogeneous gram-negative material. Discrete disc-shaped cell walls were identified in a wet mount

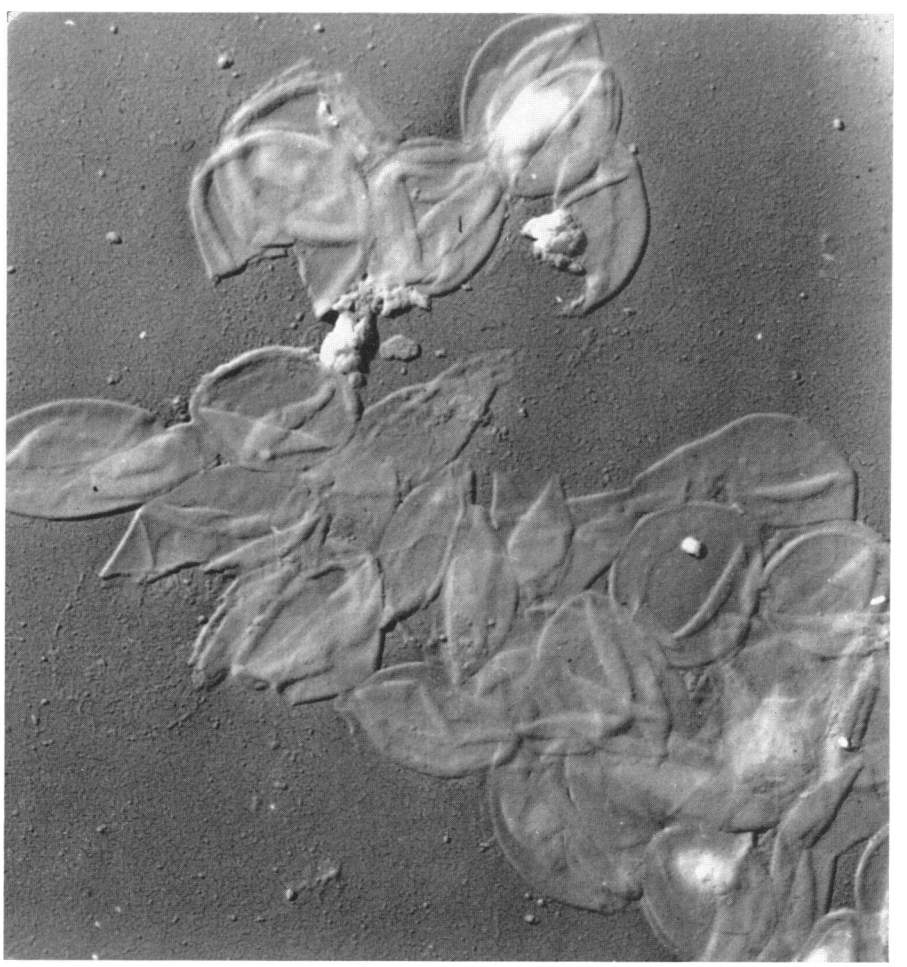

FIG. 1. *Electron micrograph of cell walls of group A-variant streptococci disrupted with a Braun homogenizer and isolated by the method of Krause and McCarthy (J. Exptl. Med. **114**:127, 1961).*

TABLE 1. *Composition of the group A-variant cell walls (strain K43 var.) after disruption by a Braun cell homogenizer*

Component	Mechanical homogenizer employed	
	Braun	Mickle*
	%	%
Rhamnose	32.3	36.0
Glucosamine	5.7	7.2
Muramic acid	3.2	5.0
Alanine	16.2	16.0
Glutamic acid	8.4	8.4
Lysine	8.7	8.8
Glycine	2.9	1.9
Serine	0.5	—

* From Krause and McCarty (J. Exptl. Med. **115**:49, 1962).

by phase-contrast microscopy. Although extensive disruption had occurred at 1.5 and 2 min, 3 min provided maximal disruption. This optimal time for disruption could vary for other bacterial species, and would be dependent upon the concentration of the bacterial suspension and the bacterial suspension–beads ratio.

Upon completion of the disruption procedure, the cell walls were separated and purified according to the method of Krause and McCarty (J. Exptl. Med. **114**:127, 1961) and lyophilized. An electron micrograph of cell walls isolated by this procedure, which includes treatment with trypsin, ribonuclease, and deoxyribonuclease, is depicted in Fig. 1. The walls are relatively free from other cellular material.

Analysis of the cell walls indicates a chemical composition similar to that obtained previously for cell walls prepared from the same strain of streptococci by the procedure which employs the Mickle disintegrator. The data in Table 1 afford a comparison between the chemical analyses for the cell walls prepared by the Braun homogenizer and by the Mickle disintegrator. No major differences in chemical composition were noted between the cell walls obtained with the use of either instrument.

Little solubilization of the cell walls occurred during the disruption procedure, as evidenced by the fact that more than 98% of the total rhamnose, initially detected in the whole bacteria, was recovered in the isolated cell walls. In this connection, it is of interest that, although traces of M protein could be detected in the fraction that did not contain cell walls after the disruption of the bacteria, by far the major bulk of this cell-wall antigen remained a component of the cell-wall fraction.

The procedure described here has also been successful for the preparation of cell walls from other bacteria, including groups A, F, and D streptococci (*S. faecalis* var. *liquefaciens*, *S. faecalis* var. *zymogenes*, and *S. durans*).

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PREPARATION OF CLEAR SILICA GELS THAT CAN BE STREAKED

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The use of silica gel media for cultivation of autotrophic nitrifying bacteria was introduced in 1891 by Winogradsky (*Microbiologie Du Sol*, Masson et Cie, Paris, 1949). Gels prepared according to earlier methods (*see* Allen, *Experiments in Soil Bacteriology*, Burgess Publishing Co., Minneapolis, 1957) were too soft to be streaked for the purification of cultures. Those gels firm enough for streaking (Temple, J. Bacteriol. **57**:383, 1949) were too cloudy for study of the minute nitrifier colonies.

Our modifications of the preparation of silica gels now permit making water-clear gels firm enough to be streaked lightly with an inoculating loop or bent glass rod. Such gels, to which appropriate mineral medium has been added, can be used for purification and for plate counts of the nitrifiers. The gels are easy to prepare and