PROTOPLAST MEMBRANE OF *STREPTOCOCCUS FAECALIS*

GERALD D. SHOCKMAN, JOSEPH J. KOLB, BOHDAN BAKAY, MARGARET J. CONOVER, AND GERRIT TOENNIES

Department of Microbiology, Temple University School of Medicine, and The Institute for Cancer Research, Philadelphia, Pennsylvania

Received for publication 10 August 1962

ABSTRACT

SHOCKMAN, GERALD D. (Temple University School of Medicine, Philadelphia, Pa.), JOSEPH J. KOLB, BOHDAN BAKAY, MARGARET J. CONOVER, AND GERRIT TOENNIES. Protoplast membrane of *Streptococcus faecalis*. J. Bacteriol. **85**:168-176. 1963.—The membrane fraction of *Streptococcus faecalis* (ATCC 9790) was isolated and purified, by a variety of procedures, from cultures that were grown under closely controlled conditions of physiological age and nutrition. The most satisfactory method required the use of lysozyme-to-cell ratios below 0.01 and the intermediate formation of protoplasts in osmotically protective media. Amino acid analyses of three of the membrane preparations indicated a characteristic and constant, but not unusual, pattern; 42% of the membranes from threonine-depleted and 49 to 55% of the membranes from log-phase cultures were accounted for as protein. Significant quantities of D-alanine or D-aspartic acid were not detected, indicating the absence of contaminating cell-wall substance. Essentially, all of the nitrogen was accounted for as amino acids. The lipid content of membranes from stationary-phase threonine-depleted (36%) and valine-depleted (40%) cultures was significantly higher than the corresponding fraction of exponential-phase cultures (28%). The phosphorus content of the membrane lipid was relatively constant (2.8 to 3.0%), and the nitrogen content was extremely low (0.12 to 0.26%). Thus, changes in the composition of the membrane fraction occurred during the transition of log-phase cells into threonine- or valine-depleted cells.

Membrane fractions (sometimes called "ghost" fractions) have been isolated from a variety of gram-positive bacteria. In some cases, the enzymatic activities of these fractions have been determined. In many of these, it is not at all clear as to whether some of these activities attributed to a "membrane fraction" are due to the protoplasmic membrane itself or to the other materials that may be isolated either in association with, or as contaminants of, the membrane fractions (for a discussion of the problems involved, *see* Weibull, Beckman, and Bergstrom, 1959). An additional problem is, of course, the isolation of the membranes without the selective loss of any of their true constituents. This is of particular concern, since Godson, Hunter, and Butler (1961) showed that their membrane preparations of *Bacillus megaterium* could be separated into two distinct macromolecular fractions by relatively gentle means, and Mitchell and Moyle (1957) found that the membrane fraction of *Staphylococcus aureus* disintegrated into small particles upon washing.

Some information is currently available about the chemical composition of the protoplast membrane fractions that have been isolated from *B. megaterium* M (Weibull and Bergstrom, 1958), *Micrococcus lysodeikticus* (Gilby, Few, and McQuillen, 1958), and *S. aureus* (Mitchell and Moyle, 1957). In general, these reports indicate that the membrane fraction represents 10 to 20% of the dry weight of the cells, is relatively rich in lipid (15 to 30% lipid), and contains 50 to 65% protein and 1 to 20% carbohydrate. In addition, the membrane fraction of *M. lysodeikticus* was found to contain a pigment, probably a carotinoid, and the lipid of the membrane was found to consist mainly of phosphatidic acids. Significant quantities of nucleic acid were not found in the membranes of either *M. lysodeikticus* or *B. megaterium* M. The presence of relatively large amounts of phosphatidic acid in the lipids from *B. megaterium*, *M. lysodeikticus*, and other bacteria was recently confirmed (Yudkin, 1962; MacFarlane, 1961*a*, *b*, 1962). It is clear that the composition of the membrane fraction of these

gram-positive bacteria differs from the cell wall and the protoplasm of the same organism.

However, there is little information concerning the consistency of composition of the membrane fraction with isolation procedure, or with environmental conditions or physiological age of the culture. Therefore, we decided to isolate the membrane fraction of *Streptococcus faecalis* (ATCC 9790) by more than one procedure after growth under precisely controlled conditions of nutrition, physical environment, and physiological age of the culture.

Membrane fractions of both rapidly growing log-phase cells and stationary-phase threonine- and valine-depleted cells were isolated. Threonine- and valine-depleted cells were selected because of previously obtained information on the chemical composition of whole cells and soluble and cell-wall fractions (Shockman, Kolb, and Toennies, 1958; Shockman, 1959; Toennies, Bakay, and Shockman, 1959). Membrane fractions were isolated with and without the intermediate formation of osmotically sensitive protoplasts, and with and without the addition of exogenous lysozyme.

Materials and Methods

Preparation of intact cells. Six large crops of cells of *S. faecalis* (ATCC 9790) were grown, harvested, and washed four times in the cold with distilled water, as previously described (Shockman et al., 1958). All cells were grown in a previously described highly buffered synthetic medium (Toennies and Shockman, 1953; Shockman et al., 1958). Threonine-depleted cells were grown in medium containing 5.4 μg/ml of threonine for 43 hr. Valine-depleted cells were grown in medium containing 7.3 μg/ml of valine for 20 hr. Both were grown to a turbidity equivalent to 0.3 to 0.5 mg/ml of cellular dry weight (Shockman, 1959). Log-phase cells were grown in complete, buffered synthetic medium to a turbidity equivalent to 1 mg/ml cellular dry weight. The required precautions were taken to be certain that these cells were still in the exponential-growth phase. These freshly grown and harvested cell crops were used for membrane isolation.

Isolation of membranes. Membrane fractions were isolated from all of the six large crops of cells. The methods used to isolate each lot of membrane fractions differed widely in general procedure as well as in detail. The general differences were as follows: (i) membrane preparations 1 and 2 were isolated after direct lysis of threonine-depleted cells with the aid of lysozyme; (ii) membrane preparation 3 was isolated after the autolysis of log-phase cells in phosphate buffer without the addition of lysozyme; and (iii) membrane preparations 4, 5, and 6 were isolated from log-phase, threonine- , and valine-depleted cells, respectively, after their conversion to protoplasts (Shockman et al., 1961*a*) with the aid of low concentrations of lysozyme, and the subsequent osmotic lysis of these protoplasts.

Thus, certain discrepancies and consistencies of the products obtained required evaluation in regard to the method of preparation. For instance, it was soon discovered that two preparations of membrane fractions (1 and 2 from threonine-depleted cells) were grossly contaminated with lysozyme. Thus, the chemical analyses of these were disregarded, and the experience gained was used to prevent similar contamination of future batches. For these reasons, the preparation of membrane fraction 5 is given in some detail, and the variations from this procedure are indicated in Table 1.

Details of the isolation of membrane preparation 5 from stationary-phase threonine-depleted cells. Threonine-depleted cells (3.4 g) were suspended in 140 ml of 0.5 M sucrose buffered with 0.05 M sodium phosphate buffer (pH 6.6); 14 mg of lysozyme (Worthington) were added, and the suspension was placed in a water bath at 37 C. The development of osmotic fragility was followed at various intervals by taking 0.1-ml samples of the incubation mixture, diluting these with 6 ml of water or the buffered sucrose solution, and reading the turbidity immediately. After 11 hr of incubation at 37 C, maximal osmotic fragility had been developed, and about 7% of the initial turbidity of the cell suspension remained after dilution with water. By use of a syringe equipped with a canula, the protoplast suspension was squirted into 500 ml of cold distilled water containing 1 mg of deoxyribonuclease that was kept vigorously agitated on a large magnetic stirrer. Lysis of the protoplast suspension was essentially instantaneous. The initial high viscosity of the lysed suspension disappeared within a few minutes. All subsequent operations were performed in the cold (0 to 5 C).

TABLE 1. *Summary of procedures used to isolate membrane fractions*

Conditions	Preparation no.					
	1. Threonine-depleted	2. Threonine-depleted	3. Log phase	4. Log phase	5. Threonine-depleted	6. Valine-depleted
Growth of cells						
Turbidity at harvest, mg/ml			0.96	1.0	0.45	0.32
Yield of cells, grams			7.3	7.0	3.4	1.30
Conditions used for isolation of membrane fraction						
Cells, mg/ml	6.0	0.6	6.5	11.7	24	26
Lysozyme, mg/ml	1.2	1.2	—	0.10	0.10	0.10
Phosphate concn M (pH 6.6)	0.20	0.20	0.01	0.05	0.05	0.05
Sucrose concn M	0.5	0.5	—	0.5	0.5	0.5
Vol of incubation mixture, ml			120	600	140	50
Lysozyme-cell ratio	0.20	2.0	0	0.009	0.004	0.004
Time*, hr	16	40	3.7	1.1	11	12
Turbidity remaining, %	ca. 15	ca. 20	14	3	7	12
No. of washings†			14	12	10	10
Yield of membrane fraction, mg			175	470	230	51
Over-all percentage	9	11	2.4	6.7	6.7	3.9
Deoxyribonuclease			−	+	+	+

* Time of incubation at 37 C for either lysis (no. 3) or protoplast formation (no. 4, 5, and 6).

† Washing procedures for preparations 3, 4, and 6 (preparation 5 given in text) were as follows. Prep. 3: four washes in buffered sucrose (0.5 M sucrose, 0.05 M sodium phosphate, pH 6.6) followed by three washes in 0.05 M NaCl, two washes with buffered sucrose, two washes in 0.1 M phosphate (Na and K), pH 7.4, and three more washes with NaCl; prep. 4: ten washes in 250 to 300 ml of 0.1 M phosphate buffer (pH 7.4) and two washes with 0.05 M NaCl; and prep. 6: six washes in 0.1 M phosphate buffer (pH 7.4), three washes in 0.05 M NaCl, and one wash in water.

The lysed protoplast suspension was then centrifuged at 25,000 × *g* for 1 hr, and the supernatant fluid was discarded. The pellet was washed by complete resuspension in 0.1 M phosphate buffer (pH 7.4) with the aid of a syringe and canula, followed by centrifugation at 25,000 × *g* for 30 min. Washing in phosphate buffer was repeated for a total of six times. For each washing, 250 to 300 ml of phosphate buffer were used. The phosphate washings were followed by three washings with 0.05 M sodium chloride and one washing with distilled water. The ultraviolet (UV)-absorption spectrum of each washing was determined. The washings showed the typical nucleic acid pattern with a 260-mμ absorption peak. The spectra showed that, although the 260-mμ peak decreased at a slow rate with each washing, a small but significant quantity of 260-mμ absorbing material was still being extracted with the last wash (Table 2). The total amount of nucleic acid in all of the washings represents less than 15% of the cellular nucleic acid.

The washed membrane preparation was then suspended in a small volume of cold distilled water and lyophilized. The over-all yield was 230 mg of purified membrane fraction or 6.7% of the cells used. Figure 1 shows electron micrographs of this preparation.

Analytical. Total nitrogen was determined, after digestion of duplicates with sulfuric acid and hydrogen peroxide (Miller and Miller, 1948), by the ninhydrin reaction (Moore and Stein, 1954), or by nesslerization. Preparation 5 gave 7.25 and 7.00% by nesslerization and 7.11 and 7.06% by the ninhydrin method.

Deoxyribonucleic acid (DNA) was determined by the method of Burton (1956) on lipid-extracted membrane preparations. Total nucleic acid was determined, after extraction with 0.5 N perchloric acid at 70 C for 15 min, from the resulting UV absorption spectrum (Toennies et al., 1959). Rhamnose was determined by the method of Dische and Shettles (1948), O-ester groups by the method of Hestrin (1949), phosphorus by the Boltz and Mellon (1947) method, amino acids by

TABLE 2. *Nucleic acid removal from membrane preparation 5*

Wash no.	Wash	Vol	OD at			Vol × OD at 260–300 mμ
			260 mμ	300 mμ	260–300 mμ	
2	PO_4*	230	0.618	0.041	0.577	133
3	PO_4	190	0.479	0.035	0.444	84
4	PO_4	230	0.302	0.025	0.277	63
5	PO_4	180	0.190	0.027	0.163	29
6	PO_4	230	0.168	0.016	0.152	35
7	NaCl†	190	0.207	0.023	0.184	35
8	NaCl	36	0.265	0.024	0.241	8.7
9	H_2O	15	1.65	1.17	0.48	7.2

* Sodium and potassium phosphates (pH 7.4, 0.1 M).

† Concentration, 0.05 M.

the semiautomatic method of Moore, Spackman, and Stein (1958), and L-amino acids and D-alanine by microbiological assay (Toennies et al., 1959; Shockman, 1962). Lipid, lipid phosphorus, and lipid nitrogen were determined on the petroleum ether-soluble fraction of methanol-extracted lipid by the procedure of Kolb, Weidner, and Toennies (1962).

RESULTS

The first two membrane preparations were made from threonine-depleted cultures, the cell walls of which are more resistant to the action of lysozyme than are those of log-phase cells (Shockman et al., 1961*a*). For this reason, relatively high lysozyme-to-cell ratios were employed (Table 1). The amino acid patterns of these two preparations were characterized by the presence of high levels of aspartic acid, arginine, lysine, and alanine (Table 3), in this regard resembling egg-white lysozyme (Block, 1956). Since the amino acid patterns were essentially identical but strikingly different from those of subsequent preparations, we have assumed that a high ratio of lysozyme to cells results in a product that is grossly contaminated if not saturated with lysozyme. Subsequently, the ratio of lysozyme to cells was kept below 0.01. This, of course, necessitated the employment of longer incubation periods to obtain complete dissolution of the cell wall (e.g., 11 hr for preparation 5). Extended exposure to 38 C might increase the risk of secondary changes (denaturation, adsorption, etc.). Therefore, it seemed worthwhile to attempt to minimize such effects by maintaining the structural integrity of the protoplasts with 0.5 M sucrose during enzyme action.

Table 4 presents the amino acid analyses of three membrane preparations (no. 3, 4, and 5) presumed to be essentially free of egg-white lysozyme. Comparison of membrane preparations 3 and 4, which were prepared from log-phase cells by different methods, shows that, although these two preparations differ little in relative distribution of amino acids, the individual values average 11% higher, and thus account for 7%

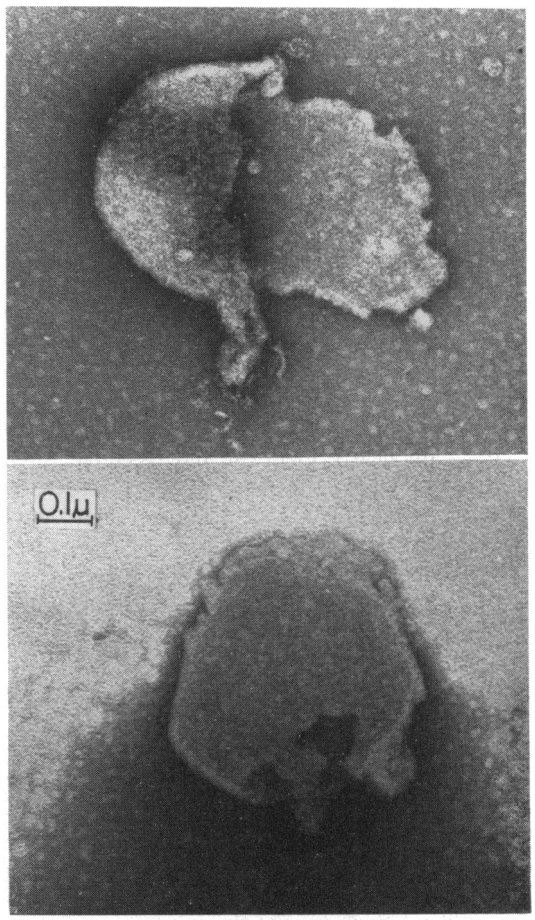

FIG. 1. *Electron micrographs of membrane preparation 5 (from threonine-depleted stationary phase cells) showing large fragments of broken membranes. The preparation was negatively stained with phosphotungstate to which a trace of sucrose had been added.*

TABLE 3. *Comparison of membrane preparations and lysozyme in regard to some amino acids**

Product	Amino acids (% of dry wt)			
	Aspartic	Arginine	Glycine	Alanine
Egg-white lysozyme	13.8	12.0	6.7	6.6
Membrane prep. 1	10.2	5.9	4.4	4.9
Membrane prep. 2	9.7	5.9	4.4	5.4
Membrane prep. 3	5.8	3.6	3.3	3.5
Membrane prep. 4	5.2	3.1	2.9	3.6
Membrane prep. 5	5.0	2.4	2.2	2.6

* Lysozyme values according to Block (1956).

more of the total membrane substance in preparation 3.

Essentially all of the nitrogen of the membrane fraction was recovered as amino acids, as shown by the agreement of the nitrogen percentage as amino acids with the total nitrogen of the membrane fractions.

Table 5 shows the results of analysis of membrane preparations 4, 5, and 6 for total lipid and for lipid phosphorus. It is clear from these results that the lipid content of the membrane fractions of threonine- and valine-depleted cells is significantly higher than that of the same fraction of log-phase cells. However, the phosphorus values of the membrane lipids (2.8 to 3.0%) are nearly identical and resemble those of the membrane fractions of *M. lysodeikticus* (Gilby et al., 1958; MacFarlane, 1961*a*) and *B. megaterium* M (Weibull, 1957). The low nitrogen content of the membrane lipid serves to confirm the figures (Table 4) for total amino acid nitrogen, which indicates that the protein component accounts for essentially all of the nitrogen of the membrane fraction of this organism.

Other substances that were determined in our membrane fractions are shown in Table 6. The nucleic acid content of preparation 4 showed reasonable agreement, as determined on UV-absorption spectra of perchloric acid hydrolysates, with the amount calculated from nonlipid phosphorus. Such was not the case for preparation 5, indicating the possible presence of some nonlipid phosphorus-containing substance other than nucleic acids.

DISCUSSION

Weibull, Zacharias, and Beckman (1959) found that radioactively labeled lysozyme was bound primarily by the cell-wall fraction, but also was bound by the soluble protoplasm and "ghost" (membrane) fractions of *B. megaterium*. At a ratio of lysozyme to cells of 0.04, 2.3% of the dry weight of their "ghost" fraction was

TABLE 4. *Amino acid analysis of membrane preparations 3, 4, and 5*

Component	Preparation no.		
	3 (Log cells, direct autolysis)	4 (Log cells, lysozyme protoplasts)	5 (Threonine cells, lysozyme protoplasts)
	%	%	%
Glutamic acid	7.0	5.8	5.8
Aspartic acid	5.8	5.2	5.0
Lysine	5.0	4.6	4.9
Alanine	3.8	3.5	2.6
Ammonia	0.8	0.9	0.7
Leucine	6.2	5.6	4.4
Glycine	3.3	2.9	2.2
Valine	5.0	4.5	3.5
Isoleucine	5.1	4.6	3.7
Arginine	3.6	3.1	2.4
Threonine	3.5	3.0	2.3
Phenylalanine	4.5	3.4	2.8
Proline	1.9	2.0	1.6
Tyrosine	3.0	2.2	1.9
Serine	2.5	2.3	2.2
Methionine	2.1	2.0	1.9
Histidine	0.8	0.9	0.7
Cystine (½)	0.5	0.2	0.6
Total	64.4	56.7	49.2
Peptide substance*	55.4	48.8	42.3
Microbiological assays			
L-Arginine	3.3	3.1	
L-Aspartic		5.9	4.6
L-Alanine	3.3	3.2	2.6
Nitrogen (from total of recovered amino acids)†	9.1	8.3	7.1
Total nitrogen	9.6 ± 0.5	8.9 ± 0.2	7.1 ± 0.1

* Total amino acids reduced by 14% (approximate correction for water loss in peptide-bond formation).

† During acid hydrolysis, tryptophan is destroyed, methionine is subject to oxidation, and tyrosine, cystine, serine, and threonine may suffer significant losses (Toennies et al., 1959). The results are given without corrections.

TABLE 5. *Lipid, lipid phosphorus, and lipid nitrogen content of membrane preparations 4, 5, and 6*

Component	Preparation no. 4 (Log phase)	5 (Threonine-depleted)	6 (Valine-depleted)
1. Lipid (% of membrane)	28.3 ± 1.4	36.0 ± 1.5	40.2 ± 0.2
Lipid P (% of membrane fraction)			
2. Determined on membrane fraction	0.84 ± 0.01	1.05 ± 0.03	1.17 ± 0.02
3. Determined on isolated membrane lipid	0.86 ± 0.02	0.95 ± 0.02	
4. Lipid P (avg of lines 2 and 3)	0.85 ± 0.03	1.00 ± 0.07	1.17
5. Lipid P (% of lipid as calculated from lines 4 and 1)	3.0 ± 0.2	2.8 ± 0.3	2.9 ± 0.1
6. Lipid N (% of lipid)	0.12 ± 0.02	0.23 ± 0.04	0.26 ± 0.02

accounted for as lysozyme. Our results (Tables 1 and 3) with *S. faecalis* indicate that a higher ratio (0.2) results in a product that is grossly contaminated with lysozyme.

The 28 and 36% lipid and 49 and 42% protein in membrane preparations 4 and 5, respectively, account for 77 and 78% of the weight of the respective preparations. Thus, the remaining 23 and 22% of membrane substance should be non-nitrogenous and nonlipid in nature. In this connection, Gilby et al. (1958) found that their membrane fractions of *M. lysodeikticus* contained an average of 19% carbohydrate as mannose, and Weibull and Bergstrom (1958) found 1 to 10% hexose as a glucose polymer in the "ghost" fraction of *B. megaterium*.

The 3% phosphorus and low nitrogen values of membrane lipid would suggest that the lipid of the membrane fraction of this organism, like that of *M. lysodeikticus* (Gilby et al., 1958; MacFarlane, 1961*a*, *b*) and *B. megaterium* M (Weibull, 1957), is constituted mainly of phosphatidic acids.

Comparison of the amino acid analyses (Table 4) of membrane preparation 4 from log-phase cells with preparation 5 from threonine-depleted cells, both prepared by the same method (lysis of lysozyme-induced protoplasts), shows two major differences: the sum of amino acids found accounts for 7.5% less of the weight of membrane in preparation 5; and, whereas in most cases the amino acid values of membrane preparation 5 are 20 to 30% lower than those of membrane preparation 4, the amounts of glutamic acid, aspartic acid, and lysine do not greatly differ. Thus, each of these three amino acids represents a significantly greater fraction of the total protein of membrane preparation 5 (e.g., glutamic acid: 11.9% of the peptide of preparation 4, 13.7% of the peptide of preparation 5). In an earlier study (Toennies et al., 1959), it was found that these same amino acids plus alanine, which together are the prominent amino acids found in the cell-wall fraction, were relatively higher in the soluble fraction of mechanically disrupted threonine-depleted cells than they were in a similar fraction of log-phase cells. These soluble fractions un-

TABLE 6. *Other substances in membrane preparations 4, 5, and 6*

Substance	No. 4	No. 5	No. 6
	%	%	%
Rhamnose	0.08 ± 0.02	0.09 ± 0.03	
O-ester groups (as acetate)	0.5	0.7	
DNA (Burton, 1956)	0.03 ± 0.02		
Total nucleic acid (UV)	4.2–5.6	1.6–2.7	0.8–1.2
P of lipid-free residue, %	0.42	0.49	
Total nucleic acid (calculated from line above)	4.2	4.9	

doubtedly contain at least some of the membrane fraction in a fragmented form. The significance of the high proportion of these amino acids in the membranes of threonine-depleted cells is not clear. The absence of significant amounts of D-amino acids (compare total aspartic acid, alanine, and arginine with L values of Table 4) seems to exclude significant contamination with cell-wall substance. However, these amino acids may be precursors of those in the cell wall, since L-amino acids seem to be the nutritional precursors of the D-amino acids of the cell wall of this organism (Shockman et al., 1961*b*).

Thus, the amino acid pattern of the membrane fractions, while not unusual, definitely differs from those previously determined on whole cells, the soluble fraction of whole cells, and the cell-wall fraction of this organism (Toennies et al., 1959).

The only other determinations of amino acid composition of a bacterial membrane fraction that we are aware of are those of Gilby et al. (1958) on membranes of *M. lysodeikticus*. Their results are expressed as molar ratios, and the amounts were estimated by paper chromatography. Our results (Table 4), calculated as molar ratios, differ widely from those of Gilby et al. For example, in their case, alanine is present in by far the highest concentrations. In ours, at least several amino acids such as glutamic acid, aspartic acid, valine, leucine, and glycine are present in molar ratios equal to or higher than alanine. Whether this is due to a true species difference or to the greater precision of the method employed here remains to be determined. Our results, which indicate that 49 and 42% of our preparations 4 and 5 respectively is protein, are in general agreement with the 50% estimated for *M. lysodeikticus* (Gilby et al., 1958), and 60 to 70% for *B. megaterium* M (Weibull and Bergstrom, 1958).

Criteria for the purity and intactness of membrane fractions remain to be established. Electron and phase-contrast micrographs are of some limited usefulness. However, visibly "pure" preparations may contain both cell-wall and protoplasmic constituents. We know of specific chemical constituents of the bacterial cell wall, such as D-amino acids, muramic acid, and rhamnose, and of the membrane, such as the lipid or lipid phosphorus (Kolb et al., 1962). These constituents seem to be quite usable as specific indicators of these anatomical components. Nucleic acids have been used as specific indicators of cellular protoplasm, but a degree of uncertainty exists. The small amounts of nucleic acid that have almost always been found with isolated membranes may or may not be true membrane components. The slow extraction of nucleic acids from the membrane preparations (Table 2) would certainly not be expected if one were dealing with simple dilution of free, contaminating nucleic acid. The amounts of nucleic acid found in our preparations seem to decrease with succeeding preparations. The small and somewhat variable amounts found in our later preparations, compared with the large amounts found in the protoplasm of this organism (Toennies et al., 1959), may not represent a structurally important component of the membrane. However, it remains difficult to decide whether this residual nucleic acid is a contaminant that is difficult to remove from the inside of a collapsed empty bag, closely bound to the membrane and possibly in the form of attached or associated ribosomes (Campbell, Hogg, and Strasdine, 1962; McQuillen, Roberts, and Britten, 1959), or is actually an important and possibly metabolically active integral part of the membrane itself. The nucleic acid in the purified membrane preparations represents, at most, 3% of the total nucleic acid originally present in the cells.

The data presented indicate the general chemical nature of the membrane fractions of *S. faecalis*, which is not quite independent of the isolation procedure employed but even more significantly is dependent on the nature of the culture from which the membrane fraction is isolated. The results indicate a change in membrane composition with the physiological age of the culture and with the nutritional condition that leads to the cessation of exponential growth. Other data, to be published elsewhere, show that the amount of membrane (as percentage of cell substance) as well as the composition of the membrane, is subject to variation in response to the nutritional environment. Changes in the amount of cell wall present under similar physiological and nutritional conditions were previously described (Shockman et al., 1958; Shockman, 1959; Toennies et al., 1959). Changes in the composition of the cell wall have not yet been thoroughly evaluated. However, the same set of nutritional conditions

seems to result in quite different effects on the cell wall and on the membrane. The mechanism by which the presence or absence of individual growth-essential amino acids, that are components of cellular proteins, exert their effects on the chemical composition of the bacterial cell and its anatomical components remains to be elucidated.

ACKNOWLEDGMENTS

This investigation was supported by research grants E-3911 and E-1935, National Institutes of Health, U.S. Public Health Service, and by a research contract (Nonr-244) from the Office of Naval Research, Department of the Navy.

The electron micrographs were prepared and taken by J. F. Anderson.

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