STUDIES OF THE BACTERIAL CELL WALL

III. PRELIMINARY INVESTIGATION OF THE CHEMICAL CONSTITUTION OF THE CELL WALL OF *STREPTOCOCCUS FAECALIS*

by

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INTRODUCTION

There has been much speculation as to the chemical constitution of the bacterial cell wall arising from studies of the wall-like structures remaining after subjection of bacteria to various treatments. One of the earliest studies of the chemical nature of the bacterial cell wall is that of VINCENZI[1], who prepared what he believed to be the cell wall ("Hülle der Zellen") of *Bacillus subtilis* by extraction with sodium hydroxide and treatment with "synthetic" gastric juice. He was unable to demonstrate the presence of cellulose; the nitrogen content of the cell wall preparations varied from 5.34–11.15%.

Cellulose, hemicellulose and chitin have been claimed as cell wall components by various investigators (see KNAYSI[2]). The possibility that chitin may be a component of the bacterial cell wall has been disputed for some time. HEIDELBERGER AND KENDALL[3] in studying the polysaccharides of Type IV pneumococcus isolated a polysaccharide with properties resembling those of chitin. They believed that this polysaccharide derived from the bacteria themselves may have accounted for the uncertainty as to whether or not chitin was a constituent of the cell wall.

KNAYSI[2] has stated that it "seems safe to conclude that the cell wall of *Acetobacter xylinum* consists mainly of true cellulose". However, the "membrane" referred to in the original investigations of BROWN[4] is more strictly a pellicle and the recent evidence of ASCHNER AND HESTRIN[5] leaves little doubt that the cellulose membrane is really a tangled mass of extra-cellular fibrils in which cells of the organism may be discerned. This has been verified in the electron micrograph of VAN ITERSON[6]. From the available experimental evidence it would seem to be an assumption to conclude that the cell wall of *Acetobacter xylinum* is itself composed of true cellulose.

Studies of the action of lysozyme have provided some indirect evidence of the nature of cell walls of susceptible organisms. EPSTEIN AND CHAIN[7] isolated from *Micrococcus lysodeikticus* a polysaccharide which they concluded was an essential element for the maintenance of the morphological structure of bacteria sensitive to lysis by lysozyme. N-acetyl amino-hexose and a substance giving all the reactions of a ketohexose were

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liberated by the action of lysozyme on the polysaccharide. HAWTHORNE[8] found that the residue remaining after dialysis of *Micrococcus lysodeikticus* lysed with lysozyme, yielded glucose and mannose and two substances tentatively identified as glucosamine and glucuronic acid on acid hydrolysis.

From a study of isolated phage receptor spots, WEIDEL[9] concluded that the cell wall, or a well-defined layer of the cell wall of *Escherichia coli* B is a phospholipoproteid of unusual properties. He was unable to demonstrate the presence of carbohydrate by liberation of reducing sugars on hydrolysis.

Cell walls may be obtained by heat-treatment rupture[10] or disintegration with minute glass beads and the procedures outlined in a previous communication[11] have provided suitable methods for preparing cell walls. This paper presents the results of an investigation of the chemical nature of the cell wall of *Streptococcus faecalis*.

METHODS

Organism. Streptococcus faecalis ST (N.C.T.C. No. 6782).

Growth conditions and harvesting. The organism was grown on a medium consisting of 3% tryptic digest of casein, 0.1% Marmite, 1% glucose and 2% agar. The surface of the medium in Roux bottles was inoculated with a few ml of overnight cultures of the organism in liquid medium. After incubation for 16 h at 37° C the cells were harvested by washing from the agar surface with distilled water, centrifugation and washing twice with distilled water on the centrifuge. Cells were finally suspended in distilled water to give suspensions containing approximately 10–20 mg dry weight bacteria/ml. Washed suspensions of cells were thus prepared from growth on 25 Roux bottles of medium on each of four different days.

Preparation of cell walls. The cell walls were prepared in four separate batches. The procedure adopted involved disintegration of cells by shaking with "ballotini" No. 12 glass beads in the Mickle disintegrator as described by SALTON AND HORNE[11]. The glass beads were removed on a sintered-glass filter, the suspensions centrifuged at 3,000 r.p.m. for 10 min to remove debris and unbroken cells followed by centrifugation of the supernatant at 10,000 r.p.m. for 10 min[11]. After repeated washings with distilled water on the high-speed centrifuge, the suspensions of each of four batches of cell walls were examined for purity in the electron microscope. Suspensions were given a final centrifugation at 3,000 r.p.m., the supernatants collected and centrifuged at 10,000 r.p.m. and the deposited cell walls freeze-dried *in vacuo* and stored separately.

Phosphorus estimation. Total and inorganic phosphorus was estimated by the colorimetric method of FISKE AND SUBBAROW[12]. Total phosphorus estimations were performed on replicate 2–3 mg samples.

Total nitrogen. Digestion of samples was carried out by the Kjeldahl method under the conditions recommended by CHIBNALL, REES AND WILLIAMS[13]. Nitrogen was then estimated colorimetrically with Nessler s reagent as described by JOHNSON[14], thus enabling a substantial reduction in the size of the initial samples. Estimations were performed on replicate samples of 5 mg, with an experimental error of $\pm$ 1.5%.

Total sulphur. The sulphur content of 0.2 g cell wall was determined by gravimetric estimation of sulphate-S after peroxide-fusion as described by LUGG[15].

Reducing sugars. Reducing power expressed as glucose was determined by the HAGEDORN AND JENSEN method (modification of HANES[16]).

Hexosamine. The colorimetric procedure of ELSON AND MORGAN[17] was used in detecting and estimating the content of hexosamine. Glucosamine — HCl was used as a standard.

Chromatographic methods

The sugars in hydrolysates were detected by paper chromatography, according to the technique described by PARTRIDGE[18]. Hydrolysates were placed on Whatman filter-paper No. 1 and run for 22 h with ethyl acetate — pyridine — water solvent (JERMYN AND ISHERWOOD[21]) and sprayed with silver nitrate — ammonia[18] or with analine hydrogen phthalate (PARTRIDGE[20]).

Identification of the amino-acids in cell wall hydrolysate was carried out by running two-dimensional paper chromatograms (Whatman filter-paper No. 1) according to the procedure of CONSDEN, GORDON AND MARTIN[22]. Phenol — ammonia and *n*-butanol — acetic acid — water (40% : 10% : 50% v/v) were used as solvents and the amino-acids were detected by spraying the papers with ninhydrin.

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RESULTS

A total of 1.5 g of cell walls was obtained from the four batches, representing a yield of 5% of the dry weight of the cells used. This yield does not represent a quantitative recovery of the cell walls. Each batch was kept separately and analysed for total phosphorus and total nitrogen contents to determine whether any marked differences between batches may be expected. The mean values of two determinations each of phosphorus and nitrogen are summarized in Table I.

TABLE I

PHOSPHORUS AND NITROGEN CONTENTS OF 4 BATCHES OF FREEZE-DRIED *Streptococcus faecalis* CELL WALL

Batch No.	% *Total P*	% *Total N*
1	1.77	5.1
2	1.76	5.4
3	1.75	5.3
4	1.77	5.2

As there appeared to be good agreement between the P and N contents of each batch, the four batches were pooled for all subsequent determinations.

GENERAL PROPERTIES

General properties

The freeze-dried cell wall preparation was subjected to a number of qualitative tests. The following reactions were obtained: Molisch test—strong positive; Biuret—weak positive; Ninhydrin test—negative; Sakaguchi test—negative; Bial reaction for pentose—negative; Acrolein test for glycerides—positive; and negative tests for reducing sugars (Fehling's solution), inorganic phosphorus and hexosamine (*p*-dimethylaminobenzaldehyde reagent).

Attempts to dissolve the cell wall preparation were completely unsuccessful. As only small amounts of the preparation (5–10 mg) could be used for solubility tests it was difficult to distinguish between complete insolubility and slight solubility. The material showed little or no solubility in the following reagents: SCHWEIZER's reagent, boiling formamide, diethylene glycol, saturated resorcinol solution, 10% trichloracetic acid, pyridine, amyl acetate, ethyl, butyl and amyl alcohols. However, both acids and alkali degraded the cell wall preparation. Material which had been heated with dilute hydrochloric acid gave strong positive tests for reducing sugar and hexosamine.

Lipoid extraction

The cell wall preparation was extracted with three successive lots of boiling anhydrous ether, each lot for 1 h. The three extracts were combined and the ether evaporated. Only a trace of material was left behind on evaporation of the ether. MILES AND PIRIE[23] found that extraction of antigenic preparations from *Brucella melitensis* with a mixture of equal parts of ethyl alcohol and ether containing 0.5% conc. hydrochloric

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acid removed lipoid which could not be extracted with ether or chloroform. 70 mg of dry cell wall preparation were extracted with successive 20 ml lots of 50:50 alcohol-ether containing 0.5% (v/v) conc. hydrochloric acid for 30 min each, at room temperature. Lipoid material amounting to 2.3% of the original dry weight of the cell wall preparation was extracted under these conditions. The extracted material was a yellow-brown greasy substance, but there was insufficient for further characterization. It seemed possible that the cell walls may contain firmly bound fatty acids, analogous to the findings of THOMAS[24] with fungi mycelium. The residue left after extraction with the alcohol-ether-hydrochloric acid solvent was therefore refluxed with 3% alcoholic potassium hydroxide, the extract acidified and extracted with ether (procedure used by THOMAS[24]). There was no evidence of additional fatty acid material having been removed from the cell wall.

Rate of hydrolysis with acid

The rate of hydrolysis with 2 *N* hydrochloric acid was followed by sealing 25 mg samples of cell wall material in glass ampoules with 5 ml acid. The sealed ampoules were heated in a boiling water bath for appropriate times, cooled by plunging into ice-cold water and immediately neutralized in the cold with sodium hydroxide. Aliquots of neutralized hydrolysates were then estimated for liberated reducing substances, hexosamine and inorganic phosphate. The results are shown in Fig. 1.

Quantitative estimations of the moisture (drying to constant weight at 105° C), ash (by incineration), total phosphorus, nitrogen and sulphur contents were determined. These data together with the percentage lipoid extracted, amounts of reducing substances, and hexosamine obtained on hydrolysis are summarized in Table II.

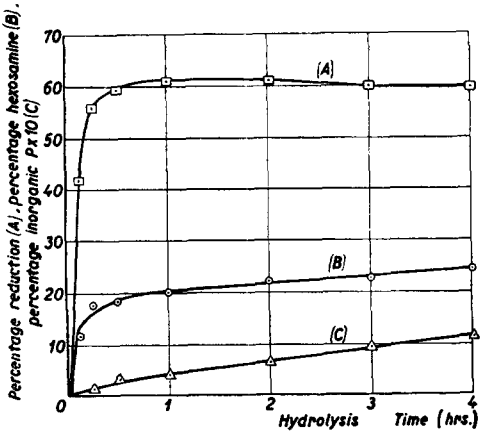

Fig. 1. Rate of hydrolysis of *Strep. faecalis* cell wall preparation by 2 *N* hydrochloric acid at 100° C, as determined by (A) percentage reducing substances, expressed as glucose; (B) percentage hexosamine, by colorimetric estimation; and (C) percentage inorganic phosphate × 10.

TABLE II

PROPERTIES OF *Streptococcus faecalis* CELL WALL

Determination	*Percentage dry weight*
Moisture	6.1
Ash	2.7
Total Phosphorus	1.88
Total Nitrogen*	5.62
Total Sulphur	0.30
Lipoid	
Ether extract	0.0
50:50 alcohol-ether-0.5% conc. HCl	2.3
Reducing substances** (expressed as glucose)	61.4
Hexosamine	22.2

* % age N — ash-free basis.

** Reducing substances and hexosamine determined after 2 *N* hydrochloric acid hydrolysis for 2 h at 100° C.

FRACTIONATION

In studying the antigenic material isolated from *Bact. dysenteriae*, MORGAN AND PARTRIDGE[26] found that the protein—polysaccharide complex could be dissociated by

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treatment with 90% phenol solutions or by aqueous alkaline solution. MORGAN AND PARTRIDGE[25] had previously shown that the protein or polypeptide portion of the complex remained as an acid insoluble residue after hydrolysis with 0.1 *N* acetic acid. PARTRIDGE[19] also demonstrated the greater solubility of the protein of cartilage mucoid on standing in 90% phenol at 37° C for 24 h. It appeared worthwhile attempting fractionation of the cell wall using these procedures, together with hydrolysis with 0.01 *N* hydrochloric acid.

Weighed amounts (50–70 mg) of cell wall preparation were subjected to the following treatments: 1. thorough mixing with 90% (w/v) phenol and allowing to stand at 37° C for 24 h; 2. extraction with 0.1 *N* sodium hydroxide for 1 h at room temperature; 3. hydrolysis with 5 ml of 0.1 *N* acetic acid for 16 h at 100° C—carried out in a sealed ampoule; 4. hydrolysis with 5 ml 0.01 *N* hydrochloric acid in a sealed ampoule for 16 h at 100° C. The extracted material was removed from the insoluble residues by centrifugation. The insoluble residues were thoroughly washed with distilled water on the high-speed centrifuge until free from extracting agents. The residues were dried *in vacuo*, and the total nitrogen and total phosphorus contents, reducing power and optical rotations of hydrolysates determined. These data are summarized in Table III.

TABLE III

ANALYSIS OF INSOLUBLE RESIDUES AFTER VARIOUS TREATMENTS OF *Streptococcus faecalis* CELL WALL PREPARATION

Treatment	*Total* N *as % dry wt.*	*Total P as % dry wt.*	*2 N HCl hydrolysis for 2 h at 100° C*	
			% Reduction	$[\alpha]_D$
Whole cell wall	5.5	1.88	61	+74°
90% phenol 24 h at 37° C	5.6	1.72	58.5	+83°
0.1 *N* NaOH 1 h at R.T.	4.85	1.61	61	+72°
0.1 *N* acetic acid hydrolysis — 16 h at 100° C	9.5	0.54	18.5	+26°
0.01 *N* HCl hydrolysis — 16 h at 100° C	9.4	0.38	37	+56°

It is evident that mild hydrolysis with acetic acid or hydrochloric acid has effected a marked degradation of the cell wall material. The acid insoluble residues which amounted to 36% (acetic acid) and 34% (hydrochloric acid) of the weight of the original material, gave much stronger positive Biuret reactions than the whole cell wall preparation. Both acetic and hydrochloric acid hydrolysates were clear, somewhat viscous solutions possessing reducing power which was further increased on hydrolysis in 2 *N* hydrochloric acid for 2 h at 100° C. However, extraction with 90% phenol and with 0.1 *N* sodium hydroxide does not appear to have substantially modified the cell wall preparation. A small amount of material (both Molisch-positive and Biuret-positive) was recovered from the dialysed phenol extract. A few milligrams of amorphous precipitate was obtained when the clear 0.1 *N* sodium hydroxide extract was adjusted to pH 4.5 with acetic acid; this material gave a strong positive Biuret reaction but was also Molisch positive.

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IDENTIFICATION OF PRODUCTS OF ACID HYDROLYSIS

For the identification of the sugars, 20 mg of the cell wall preparation was hydrolysed with 2 *N* sulphuric acid for 2 h at 100° C in a sealed ampoule. The hydrolysate was neutralized with barium hydroxide solution to pH 4.5. The barium sulphate was removed by centrifugation, washed several times and the hydrolysate and washings evaporated to dryness *in vacuo* in a glass-dish over sodium hydroxide pellets and conc. sulphuric acid. The residue was finally dissolved in 0.2 ml distilled water. The hydrolysate was then examined by paper partition chromatography, running with known sugars and spraying with aniline hydrogen phthalate. Fig. 2 shows that the hydrolysed cell wall gave "spots" corresponding very closely to D-galactose, D-glucose, and L-rhamnose. Replicate chromatograms sprayed with silver nitrate-ammonia again showed the three "spots" corresponding to D-galactose, D-glucose, and L-rhamnose.

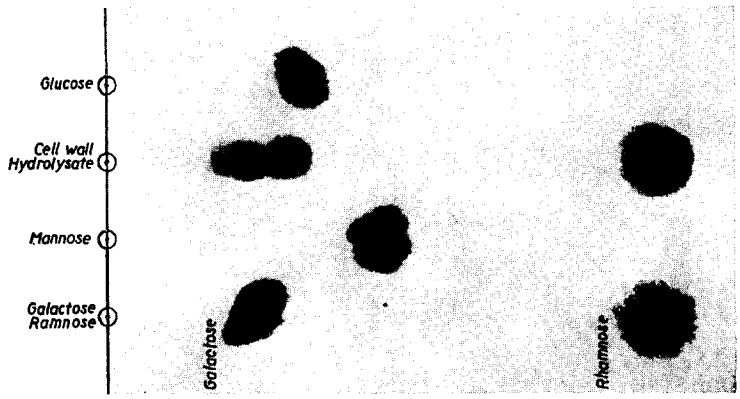

Fig. 2. Filter-paper chromatogram of cell wall hydrolysate, solutions of D-glucose, D-mannose, D-galactose and L-rhamnose. The chromatogram was run for 22 h in ethyl acetate-pyridine-water and sprayed with aniline hydrogen phthalate.

The amino-acids of the cell wall preparation were identified after hydrolysis of 7 mg cell wall with 5 ml of 5.5 *N* hydrochloric acid (in a sealed ampoule) for 36 h in a boiling-water bath. The hydrolysate was evaporated to dryness *in vacuo* over sodium hydroxide pellets and conc. sulphuric acid, re-dissolved in a little distilled water and again evaporated to dryness *in vacuo* and finally taken up in 0.3 ml distilled water. Chromatograms were run in two directions at right angles with phenol-ammonia and *n*-butanol—acetic acid—water, and sprayed with ninhydrin. Fig. 3 shows the amino-acid "spots" from the cell wall hydrolysate alone; and Fig. 4 an artificial mixture of amino-acids and glucosamine corresponding to those found in the cell wall hydrolysate. From an inspection of Fig. 3–4 it is apparent that the cell wall hydrolysate gave "spots" corresponding very closely to the following amino-acids: 1. aspartic acid; 2. glutamic acid; 3. serine; 4. glycine; 5. threonine; 6. alanine; 7. lysine; 8. valine and methionine; 9. leucine and *iso*-leucine and a "spot" corresponding to the amino-sugar; 10. glucosamine. The cell wall hydrolysate contained an additional ninhydrin-reacting substance which gave a well defined "spot" (spot 11 — Fig. 3) and possibly yet another substance (spot 12?). The substance giving "spot" 11 does not correspond to any of the amino-acids known to occur in proteins. Some samples of leucine were also found to give a "spot" in the

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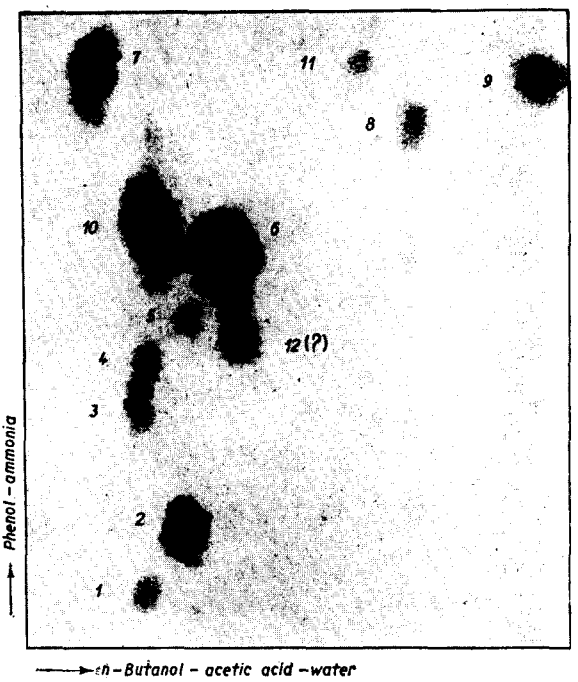
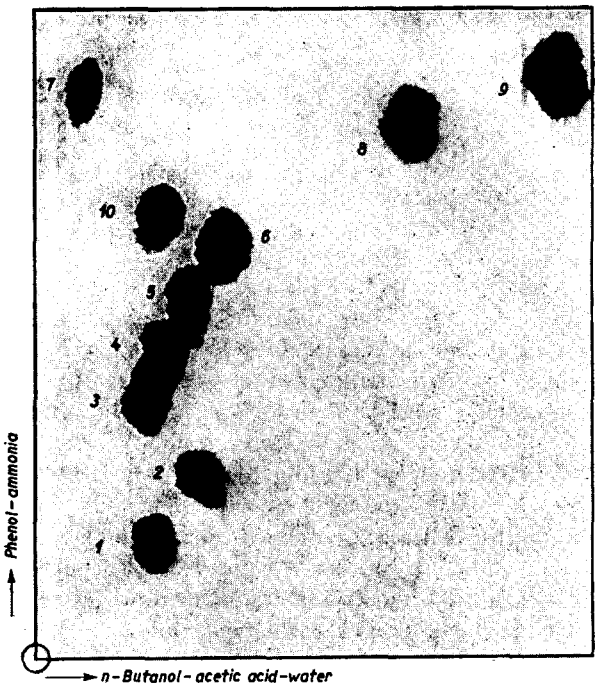


Fig. 3. Two-dimensional chromatogram of products of 5.5 *N* hydrochloric acid hydrolysis (36 h at 100° C) of *Strep. faecalis* cell wall. The hydrolysate was placed on the paper as indicated by the circle and run for 16 h with phenol-ammonia in one direction and with *n*-butanol-acetic acid -water at right angles (for 16 h). →

Fig. 4. Two-dimensional chromatogram of an artificial mixture of the following amino-acids: 1. aspartic acid; 2. glutamic acid; 3. serine; 4. glycine; 5. threonine; 6. alanine; 7. lysine; 8. valine and methionine; 9. leucine and the amino-sugar; 10. glucosamine. Chromatogram run as described for Fig. 3. ↓



same position. By running chromatograms in one direction with *n*-butanol — acetic acid — water, the unknown "spot" in the cell wall hydrolysate and that found in an impure sample of leucine did not correspond to any of the following amino-acids: α-amino-butyric acid, β-amino-butyric acid, γ-amino-butyric acid, α-amino-caproic acid, methionine, tryptophane and *iso*-leucine. As the proportion of the unknown substance to the total amino-acid complement of the cell wall preparation is so small, further identification cannot be undertaken until much larger quantities of material are available.

DISCUSSION

The fragmentary and apparently conflicting evidence of the chemical constitution of the bacterial cell wall has left little doubt as to its probable complexity. However,

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much of the available evidence has pointed to the possibility that the bacterial cell wall, or a part thereof, may be of mucoid nature. The nitrogen contents reported by VINCENZI[1], the positive tests for chitin, the isolation of a hexosamine-containing polysaccharide[3], the investigations of EPSTEIN AND CHAIN[7] and HAWTHORNE[8] are experimental findings supporting the view that the cell walls of some bacteria, at least, are composed of mucoid substances. It was therefore not surprising to find that the cell wall of *Strep. faecalis* is essentially a mucopolysaccharide.

From this preliminary investigation it is apparent that the rigid cell wall of *Strep. faecalis* gives reactions which are predominantly carbohydrate. Associated with the polysaccharide is an amino-acid containing residue. The difficulties of dissociating or differentially degrading the two components would suggest a firm chemical combination of the polysaccharide with the amino-acid residue. The stability of the cell wall complex of *Strep. faecalis* towards dissociating agents is reminiscent of the failure to separate the blood-group A substance into its polysaccharide and amino-acid moieties reported by AMINOFF, MORGAN AND WATKINS[27]. The polysaccharide component would appear to account for about 60% of the cell wall complex, but no accurate estimate of the contribution by the amino-acid residue can be made at this stage. If all the *non*-amino sugar N is "protein" N, then from the total N content of the cell wall preparation it can be estimated that there would be approximately 25% "protein" (assuming it contained 16% N). In addition to these two components, some lipoid material is present in the cell wall (approximately 2% of its dry weight), but it cannot be said to what extent this lipoid is adventitious or part of the cell wall complex.

The sugar components of the polysaccharide have been identified a glucose, galactose and rhamnose. The absence of pentose is of interest in that it substantiates the observation that no significant amounts of nucleic acid could be present in the cell wall as shown by its ultra-violet spectrum[11]. By means of paper chromatography, the amino-acids together with an amino-sugar, hexosamine, have been identified. Alanine, glutamic acid and lysine appear to be present in much greater quantities than the other amino-acids. The aromatic amino-acids and the sulphur-containing amino-acids (with the possible exception of methionine) could not be detected. The absence of the aromatic amino-acids was perhaps not surprising in view of the low absorption of the cell wall preparation at a wave-length of 280 mμ as shown in its ultra-violet spectrum[11]. One or possibly two ninhydrin-reacting substances present in the cell wall hydrolysate do not appear to correspond to any of the amino-acids commonly found in proteins.

Many more details of the constitution and structure of the bacterial cell wall will be necessary before one can decide if these rigid walls are merely inert mechanical structures. Clearly, it would be of interest to know whether the outer cell wall is completely permeable or whether it does possess some selective permeability which may be superimposed upon the permeability of the cytoplasmic membrane. It will be intriguing to see what differences there are in the chemical composition of cell walls of the Gram-positive and Gram-negative groups of bacteria. That there are differences is perhaps already suggested from the investigation of WEIDEL[9] and the findings reported here. Such studies will undoubtedly throw much light on the many physiological differences between the Gram-positive and Gram-negative organisms.

From the similarities of the chemical constitution of the cell wall complex and immunologically active polysaccharides, blood-group substances, etc., it is tempting to speculate on the role these structural walls may play in antigen-antibody reactions.

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The relationships between immunochemical analysis and cellular architecture have long been recognized and have been admirably discussed by DUBOS[28]. Indeed, much of our knowledge of the chemical nature of cell components has been derived from immunochemical studies. The preparation of a single morphological unit, such as the bacterial cell wall, would seem to offer many possibilities in integrating immunological reactions with cytological structures.

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SUMMARY

Pure cell walls of *Strep. faecalis* were prepared in four separate batches. The total P and total N contents of each batch showed sufficiently good agreement to justify pooling. A total yield of 1.5 g was obtained, representing a yield of 5% of the dry weight of the bacteria used.

1. Some general properties (including qualitative and solubility tests) of the cell wall preparation have been recorded.

2. The rate of hydrolysis of the cell wall material with 2 *N* hydrochloric acid at 100° C has been followed. The material is rapidly hydrolysed, with liberation of reducing substances, hexosamine and inorganic phosphate.

3. The cell wall contains 1.88% P; 5.6% N and 0.3% S. Lipoid material to the extent of 2.3% has been extracted. Reducing substances amount to 61.4% (expressed as glucose) on hydrolysis. The cell wall contains 22.2% hexosamine.

4. Fractionation of the cell wall complex has been attempted by treatment with 90% phenol, 0.1 *N* sodium hydroxide, and hydrolysis with 0.1 *N* acetic acid and with 0.01 *N* hydrochloric acid. Mild acid hydrolysis has effected a marked degradation in the constitution of the complex.

5. The sugars present in the cell wall preparation have been identified by paper chromatography as: galactose, glucose and rhamnose, together with an amino-sugar, hexosamine.

6. The amino-acids identified by paper chromatography are: aspartic acid, glutamic acid, serine, glycine, threonine, alanine, lysine, valine and/or methionine and leucine. Alanine, glutamic acid and lysine are present in much greater amounts than the other amino-acids. One or possibly two unknown ninhydrin-reacting substances were present in the cell wall hydrolysate.

RÉSUMÉ

Nous avons préparé des parois cellulaires pures de *Strep. faecalis* en quatre portions différentes. Les teneurs totales en P et en N de ces portions étaient suffisemment semblables pour justifier leur combinaison. Le rendement total obtenu était de 1.5 g, ce qui représente en rendement de 5% par rapport au poids sec de bactéries employées.

1. Nous avons noté quelques propriétés générales des préparations de paroi cellulaire (entre autres les résultats des essais qualitatifs et de solubilité).

2. Nous avons suivi la vitesse d'hydrolyse de la matière dont la paroi cellulaire est constituée sous l'influence d'acide chlorhydrique 2 *N* à 100° C. Cette matière est hydrolysée rapidement avec libération de substances réductrices, d'hexosamine et de phosphate inorganique.

3. La paroi cellulaire contient 1.88% de P, 5.6% de N et 0.3% de S. Nous en avons extrait 2.3% de matière grasse. A l'hydrolyse on obtient 61.4% de matières réductrices (exprimées en glucose). La paroi cellulaire contient 22.2% d hexosamine.

4. Nous avons tenté de fractionner le complexe dont la paroi cellulaire est constitué par traitement au phénol à 90%, à la soude caustique 0.1 *N* et par hydrolyse à l'acide acétique 0.1 *N* et à l'acide chlorhydrique 0.1 *N*. L'hydrolyse acide modérée eut pour effet une dégradation marquée du complexe.

5. Nous avons identifié les sucres présents dans la paroi cellulaire par chromatographie sur papier. Ce sont: galactose, glucose et rhamnose et un sucre aminé, hexosamine.

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6. Les acides aminés identifiés par chromatographie sur papier sont: acides aspartique et glutamique, sérine, glycine, thréonine, alanine, lysine, valine, et/ou méthionine et leucine. L'alanine, l'acide glutamique et la lysine se trouvent en quantités beaucoup plus importantes que les autres acides aminés. L'hydrolysat de la paroi cellulaire contenait une ou peut-être deux substances inconnues réagissant avec la ninhydrine.

ZUSAMMENFASSUNG

Reine Zellwände von *Strep. faecalis* wurden in vier verschiedenen Ansätzen hergestellt. Die Gesamtgehalte an P und N der verschiedenen Ansätze stimmten genügend überein um deren Vereinigung zu rechtfertigen. Die Gesamtausbeute betrug 1.5 g, das ist 5% des Trockengewichtes der verwendeten Bakterien.

1. Einige allgemeine Eigenschaften der Zellwandpräparate (mit Inbegriff von qualitativen und Löslichkeitsversuchen) wurden verzeichnet.

2. Die Hydrolysegeschwindigkeit des Zellwandmaterials unter dem Einfluss von 2 *N* Salzsäure bei 100° C wurde verfolgt. Das Material wird schnell hydrolysiert wobei reduzierende Substanzen, Hexosamin und anorganisches Phosphat in Freiheit gesetzt werden.

3. Die Zellwand enthält: 1.88% P, 5.6% N und 0.3% S. 2.3% Lipoide wurden extrahiert. Die bei der Hydrolyse entstehenden reduzierenden Substanzen betrugen 61.4% (ausgedrückt als Glucose). Die Zellwand enthält 22.2% Hexosamin.

4. Die Fraktionierung des Zellwand-Komplexes wurde mit 90%-igem Phenol mit 0.1 *N* Natriumhydroxyd und durch Hydrolyse mit 0.1 *N* Essigsäure und 0.1 *N* Salzsäure versucht. Die schwach saure Hydrolyse hatte einen bedeutenden Abbau des Komplexes zur Folge.

5. Die Zucker des Zellwand-Präparates wurden durch Papierchromatographie identifiziert. Es sind: Galactose, Glucose, Rhamnose und ein Aminozucker, Hexose.

6. Die mit Hilfe der Papierchromatographie identifizierten Aminosäuren waren: Asparagin- und Glutaminsäure, Serin, Glycin, Threonin, Alanin, Lysin, Valin, und/oder Methionin und Leucin. Alanin, Glutaminsäure und Lysin waren in viel grösseren Mengen vorhanden als die anderen Aminosäuren. Eine oder möglicherweise zwei unbekannte Substanzen welche mit Ninhydrin reagieren, waren in dem Zellwand-Hydrolysat vorhanden.

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