were adjusted so that there would be virtually no inhibition of transport at pH 7.8. This demonstrates that the lesser transport of the dibasic substances under conditions of high pH may not be ascribed to toxicity of these detergent-like substances because the transport of the naturally occurring bile salts was not adversely affected. The foregoing data are in accord with our hypothesis that the ileal transport system for bile salts is specific for cholanic acid derivatives containing a single negative charge on the side chain.

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Release of lipopolysaccharide during the preparation of cell walls of *Pseudomonas aeruginosa*

At alkaline pH values EDTA has a potent bactericidal action against *Pseudomonas aeruginosa*[1] and causes release of lipopolysaccharide from the isolated cell wall of the organism[2]. During a study of the probable connection between the two effects it was desirable to estimate the contribution made by the cell wall to the mass of the whole cell. For Gram-negative bacteria a figure of 20% or less is generally accepted[3], although a doubtfully high value of 76–78% was indicated by early work[4] on *P. aeruginosa*. These values have generally been obtained either directly, from the yields of cell walls, or by calculation, using nitrogen analyses of soluble and insoluble fractions of cells.

In the present work, analyses of washed whole cells and of isolated cell walls of *P. aeruginosa* (NCTC 1999) have been made for compounds believed to be uniquely or predominantly present in either the lipopolysaccharide or the glycosaminopeptide fraction of the cell wall. Walls were prepared from cells grown for 24 h at 37° on Tryptone glucose extract agar (Oxoid) and disintegrated using a Braun MSK

homogeniser, and were treated with trypsin and ribonuclease[1]. Water-washed cells and purified cell walls were lyophilised and dried *in vacuo* over P_2O_5 for analysis. Amino acid and amino sugar contents were determined using a Technicon auto-analyser after hydrolysis of samples in 6.1 M HCl at 105° for 16 h. Because of extensive destruction of amino sugars under these conditions, analyses were also made

TABLE I

ANALYSIS OF WHOLE CELLS AND CELL WALLS OF *P. aeruginosa*

Component	*Occurrence in cell wall*	*Hydrolysis period (h)**	*Composition (%)*		*Apparent contribution of cell wall to mass of cell (%)* $\left(\frac{100C}{W}\right)$
			Cell wall (W)	*Whole cell (C)*	
Diaminopimelic acid	Glycosaminopeptide	16	1.91	0.273	14.3
Muramic acid	Glycosaminopeptide	4	2.11	0.333	15.8
		16	1.38	0.232	16.8
Glucosamine	Glycosaminopeptide, lipopolysaccharide, lipid	4	2.84	0.633	22.3
		16	2.16	0.518	24.0
Galactosamine	Lipopolysaccharide	4	1.03	0.383	37.2
		16	0.622	0.182	29.3 (36.4)**
Hydroxy acids***	Lipopolysaccharide	2	4.89	1.58	32.3

* Hydrolysis at 105° using aqueous HCl; 6.1 M for amino compounds, 3 M for hydroxy acids.

** Result in parenthesis obtained by method of CESSI AND SERAFINI-CESSI (ref. 16).

*** Total acids determined using a palmitic acid standard.

on 4-h hydrolysates. For the colorimetric estimation[5] of long-chain hydroxy acids, samples were hydrolysed for 2 h at 105° in 3 M HCl, and fatty acids were extracted from the hydrolysates using petroleum ether; hydroxy acids were separated from other acids by chromatography on silicic acid in a micro modification of the method of O'BRIEN AND ROUSER[6].

Calculations based on the results of these analyses (Table I) showed that when lipopolysaccharide components[7,8] were used as markers for the cell wall, the latter appeared to account for a significantly larger proportion of the cell mass than when glycosaminopeptide components were used. This could be explained either by a selective loss of lipopolysaccharide[9] from the cell wall during its isolation, or by the occurrence elsewhere in the cell of substantial amounts of the components being estimated. We are not aware of another major source of galactosamine, and although glucosamine is present in the lipids of the cell wall and/or protoplast membrane[2,10,11], the membrane probably accounts for less than 5% of the total glucosamine of the cell. 3-Hydroxymyristic acid, a characteristic component of lipopolysaccharide from other bacteria[12], was absent from *P. aeruginosa*; the components of the hydroxy acid fractions from both whole cells and cell walls were provisionally

identified by gas–liquid chromatography of their methyl esters as 3-hydroxydecanoic, 2-hydroxydodecanoic and 3-hydroxydodecanoic acids. The 3-hydroxy acids occur in extracellular rhamnolipids produced by *P. aeruginosa*[13] and have recently been independently identified[14] as components of lipopolysaccharide from this organism. In agreement with other workers[14,15] we did not detect hydroxy acids in lipids extracted from whole cells by neutral or acidified chloroform–methanol mixtures at room temperature.

To substantiate the apparent loss of lipopolysaccharide, various supernatant fractions obtained during the collection and purification of crude cell walls by differential centrifugation were examined for the presence of lipopolysaccharide and glycosaminopeptide components. There was clear evidence in each case for a selective solubilisation of lipopolysaccharide and protein relative to glycosaminopeptide; materials containing glucosamine and galactosamine were present in the initial supernatant fluid containing intracellular solutes and fragments of membrane, *etc.*, in aqueous washings and in a trypsin–ribonuclease digest of the walls. Similar amounts of lipopolysaccharide components were present in a control digest without added enzymes. Selected fractions were also analysed for hydroxy acids and for materials reacting with thiobarbiturate, after periodate oxidation, to produce a chromogen with an absorption maximum at about 550 mμ (ref. 17). Such material was assumed to be the 2-keto-3-deoxyaldonic acid component of lipopolysaccharide. As shown in Table II, the relative amounts, in supernatant fractions, of components expected to be specific to lipopolysaccharide were closely similar to those in whole cells and purified cell walls. Similar ratios have been obtained for lipopolysaccharides isolated both from acetone-dried cells and isolated cell walls[8]. Thus, the supernatant fractions appear to contain lipopolysaccharide, but little or no glycosaminopeptide, arising from the cell wall. It is not yet clear whether the lipopolysaccharide released differed from that remaining in the material designated purified cell wall, nor has the mechanism of its release been studied. As lipopolysaccharide continued to be released after extensive washing of the walls, the results are unlikely to be explained by the existence of fragile fibrils of lipopolysaccharide-containing antigen extending outwards from the cell surface[18]. It seems more likely that there is a slow dissociation of lipopolysaccharide and protein, perhaps as a result of autolytic modification to the cell wall.

TABLE II

RELATIVE AMOUNTS BY WEIGHT OF LIPOPOLYSACCHARIDE COMPONENTS

Sample	*Galactosamine**	*Hydroxy acids*	*2-Keto-3-deoxyaldonic acid***
Whole cells	1.0	8.6	—***
Cell walls	1.0	7.8	1.8
Supernatant fractions†	1.0	8.0	2.2

* Using results from 16-h hydrolysates.

** Calculated as 2-keto-3-deoxyoctonic acid.

*** Estimation not possible because of interference by intracellular materials.

† From suspensions of cell walls (previously washed several times using deionised water and 1 M NaCl at 4°) after incubation for 3 h at 37° in phosphate buffer pH 7.6, with or without added enzymes.

Although no accurate assessment for the contribution of the cell wall to the mass of the cell has been possible, it may be appreciably larger than the value of about 15% suggested by analyses for glycosaminopeptide components using trypsin-treated walls. It is also apparent that the extent of action by EDTA on whole cells of *P. aeruginosa* cannot be properly assessed from experiments with isolated cell walls. These results and those of CORPE AND SALTON[9] emphasise the need for caution when using cell walls for the study of cell surfaces and particularly as a source of lipopolysaccharide.

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Effect of ouabain on deoxynucleoside metabolism in hereditary spherocytic human erythrocytes

The active movements of Na^+ and K^+ in the human erythrocyte are now known to be coupled to the activity of an ATP-hydrolyzing system (ATP phosphohydrolase, EC 3.6.1.4) located in the membrane, which is stimulated synergistically by Na^+ and K^+ (refs. 1, 2). Inhibition of active cation movements by the cardiac glycoside, ouabain causes a fall of 15% in the glycolytic rate when fresh erythrocytes are incubated *in vitro*[3], thus demonstrating that the activity of the membrane