TABLE I

GLYCOLIC ACID CONTENT OF FRACTIONS ISOLATED FROM HOG PANCREAS

	Glycolic acid (%)	*N-Glycolylsialic acid (%)*
Lyophilized material	14.0	60
Fraction A	14.6	62
Fraction B	15.0	64

Glycolic acid determined by the procedure of KLENK AND UHLENBRUCK[6].

The results are given as percentages of the sialic acid content determined by the resorcinol reaction.

Since the yield of the sialic acids was rather low, enrichment of one type of sialic acid must be considered. However, the liberation of the two sialic acids went parallel, as judged from the paper-partition chromatograms, and during the crystallization of the sialic acids, no significant change in the ratio of the two sialic acids occurred (see Table I).

The present results indicate that the mucoids of the secretory glands in the hog have a much higher percentage of N-glycolylsialic acid than the glycoproteins of the serum-protein type. The percentage of the glycolyl-containing sialic acid was lower in the pancreas than in the submaxillary gland. The pancreas secretion is, however, more serous, and the mucoid concentration is rather low in comparison with that in the submaxillary gland. As the amount of glycoproteins of the serum type probably is the same in the two organs, a lower glycolyl content in the sialic acids of hog pancreas is to be expected if the hypothesis is valid.

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An effect of drying the isolated cell walls of *Streptococcus faecalis* and a *Pseudomonas* species

Extraction of acetone-dried bacterial cells with cold 5% TCA* is a method in general use for obtaining preparations of surface polysaccharides and related complexes. In an investigation to be reported elsewhere, cytoplasmic constituents of *Streptococcus faecalis* (N.C.T.C. No. 6782) and a species of *Pseudomonas* (described previously[1]) were being examined in TCA extracts of acetone powders and it became necessary to learn whether or not any cell-wall components might appear in the extracts. It seemed probable that a surface material, analogous to the so-called "O" somatic antigen of other Gram-negative bacteria, was being removed from the *Pseudomonas* but the strain of *S. faecalis* was not known to produce any such component. In the Gram-negative bacteria the relationship between the surface polysaccharides and the structural components of the rigid cell wall is uncertain and the effect of TCA on the isolated cell walls of both Gram-negative and Gram-positive bacteria has not been investigated.

Cell walls were prepared from stationary-phase cells by Mickle disintegration[2] followed by tryptic digestion, washing and, where applicable, freeze-drying. Cell-wall preparations (15–50 mg) were extracted overnight at 3° with TCA (5% w/v, 20 ml) or water (20 ml) and centrifuged off. TCA-extracted cell walls were washed once with cold 5% TCA (20 ml), 3 times with water and freeze-dried. Water-extracted cell walls were washed once with cold water (20 ml) and freeze-dried. Monosaccharide constituents of the various preparations were detected after hydrolysis for 2 h at 100° with 2 *N* HCl by paper chromatography in butan-1-ol–acetic acid–water, 6:1:2 and butan-1-ol–ethanol–water, 5:1:4 (top layer) and reaction with *p*-anisidine hydrochloride or

* Abbreviations used are; DAP, *α,ε*-diaminopimelic acid; DNP-, 2,4-dinitrophenyl-; FDNB, 1-fluoro-2,4-dinitrobenzene; TCA, trichloroacetic acid.

TABLE I

EXTRACTION OF FREEZE-DRIED CELL WALLS WITH TCA

	S. faecalis			*Pseudomonas*		
	Wall	*Extract*	*Residue*	*Wall*	*Extract*	*Residue*
Yield (% of cell wall)		5–10	90–95		10–30	70–90
Solubility in H_2O after removal of TCA		Soluble	Insoluble		Insoluble	Insoluble
Retention by dialysis membrane		Retained			Retained	
Composition*	Glucose Galactose Rhamnose Hexosamine Muramic acid Alanine Glutamic acid Lysine plus traces of other amino acids	Glucose** Galactose** Rhamnose Hexosamine (Glutamic acid)** plus traces of other amino acids	Glucose Galactose Rhamnose Hexosamine Muramic acid Alanine Glutamic acid Lysine (plus traces of other amino acids***)	Glucose Rhamnose Hexosamine Muramic acid DAP plus "typical" protein amino acids	Glucose Rhamnose Hexosamine plus traces of "typical" protein amino acids	Glucose Rhamnose Hexosamine Muramic acid DAP plus "typical" protein amino acids
N-Terminal amino acids§	Alanine	Alanine	Alanine	Aspartic acid Glutamic acid Glycine Alanine	Aspartic acid Glutamic acid Glycine Alanine	Aspartic acid Glutamic acid Glycine (? trace) Alanine

* The effect of the treatment on lipid materials was not examined but the greater ease of wetting the *Pseudomonas* cell wall after extraction suggests that lipid was removed.

** The yield and composition of the extract varied. Glucose, galactose and rhamnose were evidently all present in some cases but in 2 preparations the only sugars which could be detected in significant quantity were galactose and rhamnose. Similarly glutamic acid was not always present and when it did appear it was in low proportion.

*** In 2 preparations these "trace" amino acids (mainly aspartic acid, valine/methione and leucine/isoleucine) were completely removed by the TCA solution. In a third preparation detectable quantities remained in the extracted residue.

§ The constituents present in highest proportion are underlined.

aniline phthalate. Amino acids and amino sugars were detected after hydrolysis for 16 h at 105° with 6 *N* HCl by 2-dimensional paper chromatography in pyridine–water, 4:1 followed by butan-1-ol–acetic acid–water, 6:1:2 and reaction with ninhydrin. DAP was detected[3] specifically by paper chromatography in methanol–water–10 *N* HCl–pyridine, 160:35:5:20. N-terminal amino acids were detected as their DNP-compounds after reaction with FDNB[4].

Table I gives results obtained with TCA but it was also found that cold water removed up to 10% of the weight of the dried cell walls in a form which had similar qualitative composition of amino acids, amino sugars and monosaccharides to the TCA extracts. The N-terminal amino acids in water extracts were not determined. Differences, possibly in lipid composition, between the two types of extract of the *Pseudomonas* cell walls may be inferred from the fact that removal of the TCA with ether from the TCA extract caused the latter to become turbid. The water extract was faintly opalescent.

It has been suggested that the amino acids other than the major cell-wall components of Gram-positive bacteria might be derived from cytoplasmic material which remains in close association with the cell-wall preparations[5]. To some extent, the results of extracting dried cell walls with TCA support this belief. Such treatment does not alter basic qualitative composition but chromatograms of the hydrolysed residues of some preparations of *S. faecalis* gave with ninhydrin no trace of any substance other than the two amino sugars and three main amino acids. The failure of TCA to effect complete removal of the trace amino acids in every case remains unexplained but might be due, in part, to variations in purity of the different cell-wall preparations. It is probably also true that contaminating material is removed from the *Pseudomonas* cell wall (the residue is lighter in colour than the untreated cell wall) but the extent to which this occurs is difficult to assess because of the greater number of amino acids present. Similarly it is difficult to decide whether the high proportion of N-terminal glycine in the TCA extract of the *Pseudomonas* cell wall was derived from the wall itself or from contaminating material. It is of some interest that no DAP was detected in any of the *Pseudomonas* extracts.

If the cell walls were not dried before treatment, neither water nor TCA had any appreciable effect. If, on the other hand, the washed residues were again freeze-dried, further treatment with either solvent removed more material. How many times this process can be repeated has not been ascertained. It would seem, therefore, that some of the changes occurring on drying the cell wall are irreversible, at least over relatively short periods after rewetting. It is possible that hydrogen-bond rearrangement which occurs on drying together with changes in less specific bonding forces expose to solvent action sites which were inaccessible while the cell wall was fully hydrated. Differences in drying conditions might account in part for differences in yield and apparent composition of the TCA extracts noted in Table I and it does not follow that any material extracted in this way from acetone-dried cells will necessarily be the same as that obtained from freeze-dried cell-wall preparations. Materials broadly resembling those described in Table I, however, have been found in TCA extracts of acetone-dried cells of both organisms.

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Preliminary Notes

On the biosynthesis of heme and hemeproteins in liver cell

The synthesis of heme in the immature or nucleated red cell has been studied extensively by SHEMIN, RIMINGTON and other workers, with the result that the mechanism of porphyrin biosynthesis is now known. The biosynthesis of the tissue hemeproteins which play an important role in the respiration of aerobic cells, has been studied less extensively. THEORELL *et al.*[1] purified various hemeproteins from different organs after intraperitonal injection of radioactive iron into the guinea pig and studied the rate of its incorporation. DRABKIN[2] observed the incorporation of ^{14}C-labelled glycine into cytochrome *c* by liver slice. These authors concluded that heme synthesis occurred in the individual aerobic cells.