Appendix 8. Rumen-fluid, carbohydrate medium
(after Bryant & Robinson, 1961).

Peptone	0.1g
Tryptone	0.1g
Yeast extract	0.05g
Salts solution A	7.5ml
Salts solution B	7.5ml
Indigo carmine (0.05%)	1.0ml
Rumen fluid	30.0ml
Sodium bicarbonate	0.05g
Cysteine	0.05g
Galactose	0.05g
Maltose	0.05g
Lactose	0.05g
Glucose	0.05g
Agar	1.5g
water to make	100ml

Prepared as described under pre-reduced media. Rumen fluid was obtained from a local abbattoir, filtered through cheesecloth into bottles purged with oxygen-free gas for storage. Small quantities were clarified by centrifugation at 12 000 *g* before incorporation into culture media.

Appendix 9 Lowry's method for proteins

(Lowry et al., 1951)

Reagents

1) 2% sodium carbonate in 0.1 normal sodium hydroxide.
2) 0.5% copper sulphate in 1% sodium potassium tartrate.
3) Solutions mixed before use, 5ml of 1 with 0.1ml of 2.
4) Folin and Ciocalteu's reagent, 5ml diluted to 12ml in distilled water

Standard

Crystalline human albumen in dilutions from 1.0 mg ml^{-1}

Test

1) 0.4ml of samples pipetted into test tubes,
2) 2ml of copper reagent added and stood for 10 min,
3) 0.2 ml of diluted Folin's reagent added and stood for 30 min,
4) Read in an absorptiometer at 500nm,
5) Protein values calculated from standard curve.

Appendix 10. Determination of hexoses with Anthrone

(Siefter et al., 1950).

Reagents

2% Anthrone (Merck) in 95% sulphuric acid in water, made up freshly before use.

Standard.

Glucose solution, in dilutions from 20µg ml^{-1}

Test

1) 1.0ml of sample pipetted into a tall tube,
2) 2.0ml of anthrone added quickly but carefully,
3) tubes placed in a boiling water-bath for 10 min, and cooled in water.
4) Read in an absorptiometer at 620nm.

Total hexoses as single sugars and in glycoconjugates were calculated from standard curve.

Appendix 11. Turbidity test for sulphates

(Lloyd, 1966).

Reagents.

M hydrochloric acid

0.5% barium chloride in 0.5% gelatin, made up freshly.

0.5% gelatin solution.

4% trichloroacetic acid

Standard

potassium sulphate, 500 $\mu g\ ml^{-1}$.

Test

1) Samples were hydrolysed in 1.0ml of M HCl at 85°C for 2 h then dried under vacuum.
2) Residue dissolved in 1.0ml of distilled water.
3) Two tubes were set up for each hydrolysate, and standard.
4) 0.1ml added to each of two tubes plus 1.4ml of TCA soln.
5) To one tube was added 0.5ml of gelatin soln, to the other, 0.5ml of $BaCl_2$/gelatin.
6) All tubes stood at ambient temperature for 30 min.
7) Read in a absorptiometer at 360nm.

Sulphate concentration derived by subtracting OD of gelatin from OD of barium/gelatin readings and calculation from standard values.

Appendix 12. Glycoproteins by the Periodic acid-Schiff's method
(Mantle & Allen, 1978).

Reagents.

Periodic acid, 50% solution. 10µl were added to 10ml of 7% acetic acid.

Schiffs reagent.

Basic fuchsin (BDH), 1.0g added to 100ml hot water. 20ml of M HCl added plus 1.7g of sodium metabisulphite. When all was dissolved, 0.3g activated charcoal added. After standing overnight, the clear leuco-dye solution was filtered off and stored at 4°C.

Standard.

Glycogen, 1mg ml^{-1} was used to confirm that test conditions were satisfactory, but not used to quantitate glycoproteins, because of their heterogeneity.

Test

1) 1.0ml Glycoproteins in solution precipitated with 1.5ml ethyl alcohol (final concentration, 60%).
2) Deposit collected from centrifugation and re-dissolved in 2.0ml of 0.2M NaCl,
3) 0.2ml of fresh periodate added and incubated at 37°C for 2 h,
4) 0.2ml Schiff's reagent added,
5) Stood for 30 min for colour development and
6) read at 550nm and optical densities recorded.

The colour obeyed Beer's law with glycogen and mucin (Figure 19) but gave no reaction with single hexoses. All glassware had to be thoroughly clean to avoid oxidation of the Schiff's reagent.

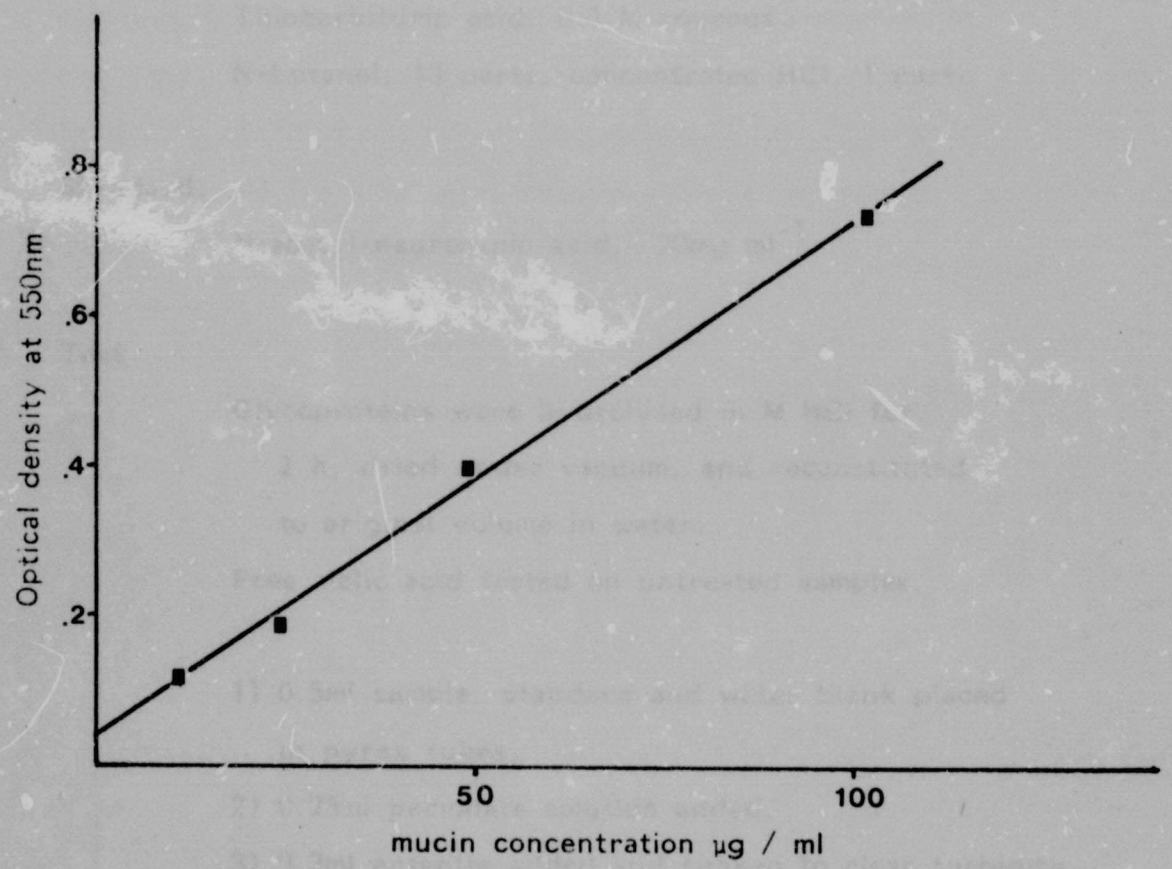

Figure 19. Graph of concentration of pig gastric mucin plotted against the optical density at 550nm of dilutions analysed by the Periodic acid Schiffs method

Appendix 13. Determination of sialic acids

(Warren, 1959, modified by Codington et al;, 1976).

Reagents.

Periodic acid, 0.025 M in 0.125 N sulphuric acid.

Sodium arsenite, 2% in 0.5 M hydrochloric acid.

Thiobarbituric acid, 0.1 M aqueous.

N-butanol, 19 parts, concentrated HCl, 1 part.

Standard.

N-acetyl-neuraminic acid, 20mg ml^{-1}

Test

Glycoproteins were hydrolysed in M HCl for 2 h, dried under vacuum, and reconstituted to original volume in water.

Free sialic acid tested on untreated samples.

1) 0.5ml sample, standard and water blank placed in pyrex tubes,
2) 0.25ml periodate solution added,
3) 0.2ml arsenite added and shaken to clear turbidity,
4) 2.0ml thiobarbituric acid added to mixture,
5) tubes placed in boiling water for exactly 7.5 min,
6) 4 ml of solvent mixture added to cooled tubes which were shaken and centrifuged.
6) solvent phase collected and read at 549nm in glass cuvettes.

After calculation of sialic acid concentrations from standard values, subtraction of free acid from total acids gave the value of sialic acids bound to the glycoprotein molecule.

Appendix 14. P-nitrophenol glycoside calibration

Reagents

P-nitrophenol, 10 µM / ml (Sigma)

Test

Develcpment of the standard enzyme test is given in section 2.14, and the procedure detailed in section 2.14.6. To calculate the amount of p-nitrophenol released from enzyme reactions, the stock reagent was diluted two-fold and used as the substrate component in the standard test. The graph below (Figure 20) illustrates the plot of p-nitrophenol concentration and optical density at 410 nm.

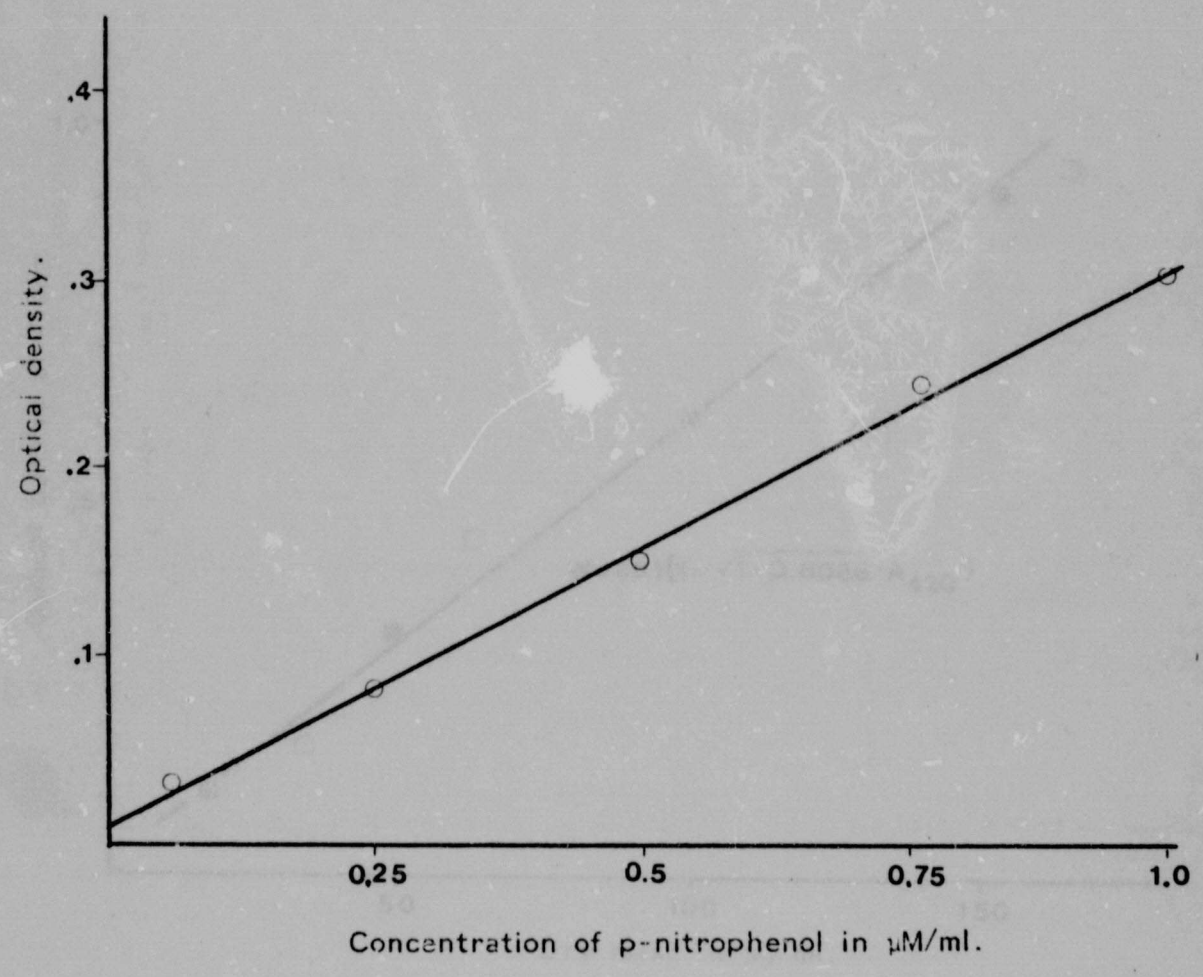

Figure 20. Graph for p-nitrophenol concentration when dilutions of stock solution (Sigma) were tested in the standard enzyme assay and plotted against optical density at 410 nm

Appendix 15. Calculation of dry mass in bacterial cultures from optical density

(Koch, 1981)

Experimental procedure.

Detailed procedure for calculation of dry mass is given in section 2.12. Cell pellets were dried in previously weighed optically-calibrated tubes and the dry mass calculated by subtraction. The graph below illustrates the relationship between mass and optical density at 410 nm. Also included on the graph is the theoretical plot of dry mass and optical density derived from the formula given by Koch (1981). (Figure 21).

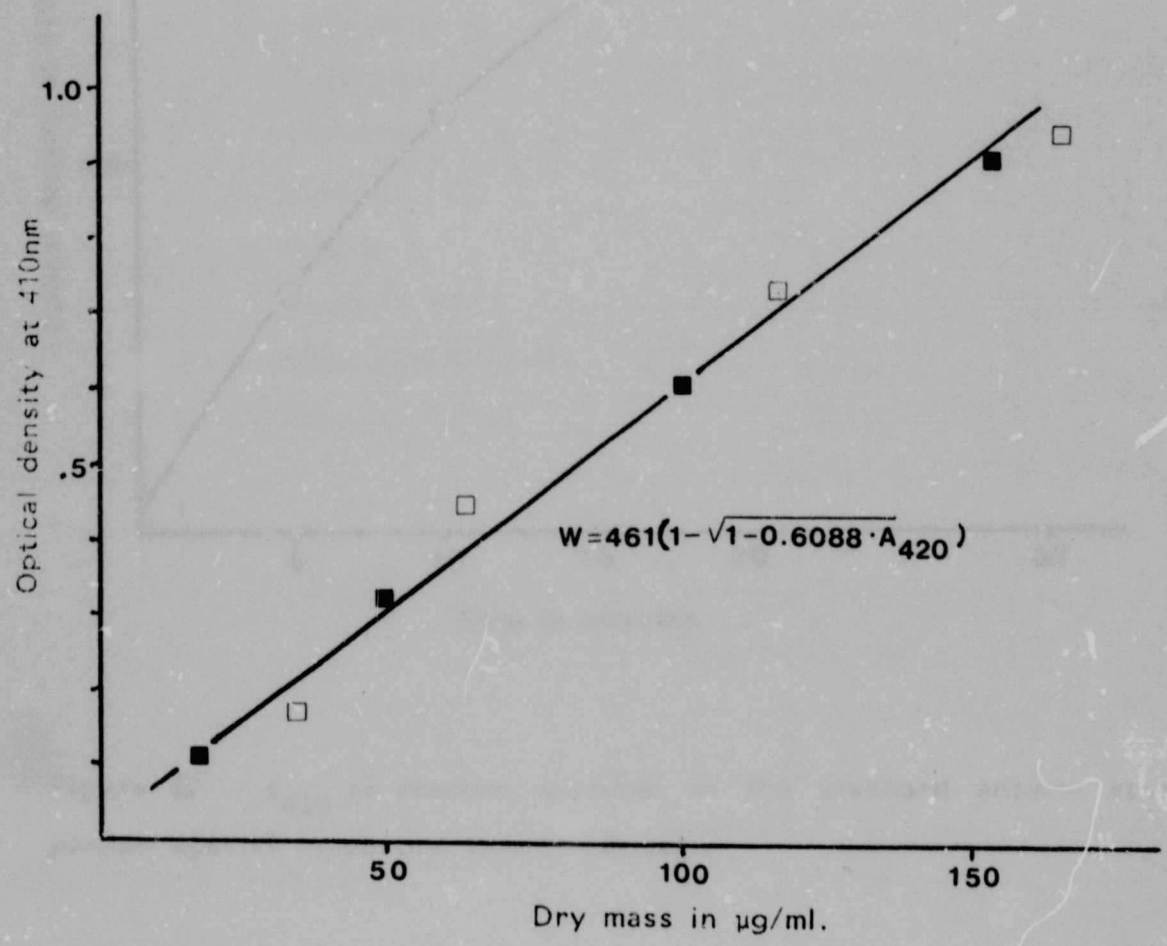

Figure 21. Theoretical graph for dry mass determination (Koch, 1981): ■—■, and data plotted from experimental dry mass determinations: □—□.

Appendix 16. Determination of optimum incubation time for enzyme assays.

β-galactosidase was assayed in the standard test as described in section 2.14.6 and incubated for 5, 10, 15, 20 and 30 minutes. The A_{410} of each reaction mixture was read after addition of 2 ml of sodium carbonate and plotted against the incubation time (Figure 22). A time of 15 minutes was chosen to ensure that the rate of substrate release was linear.

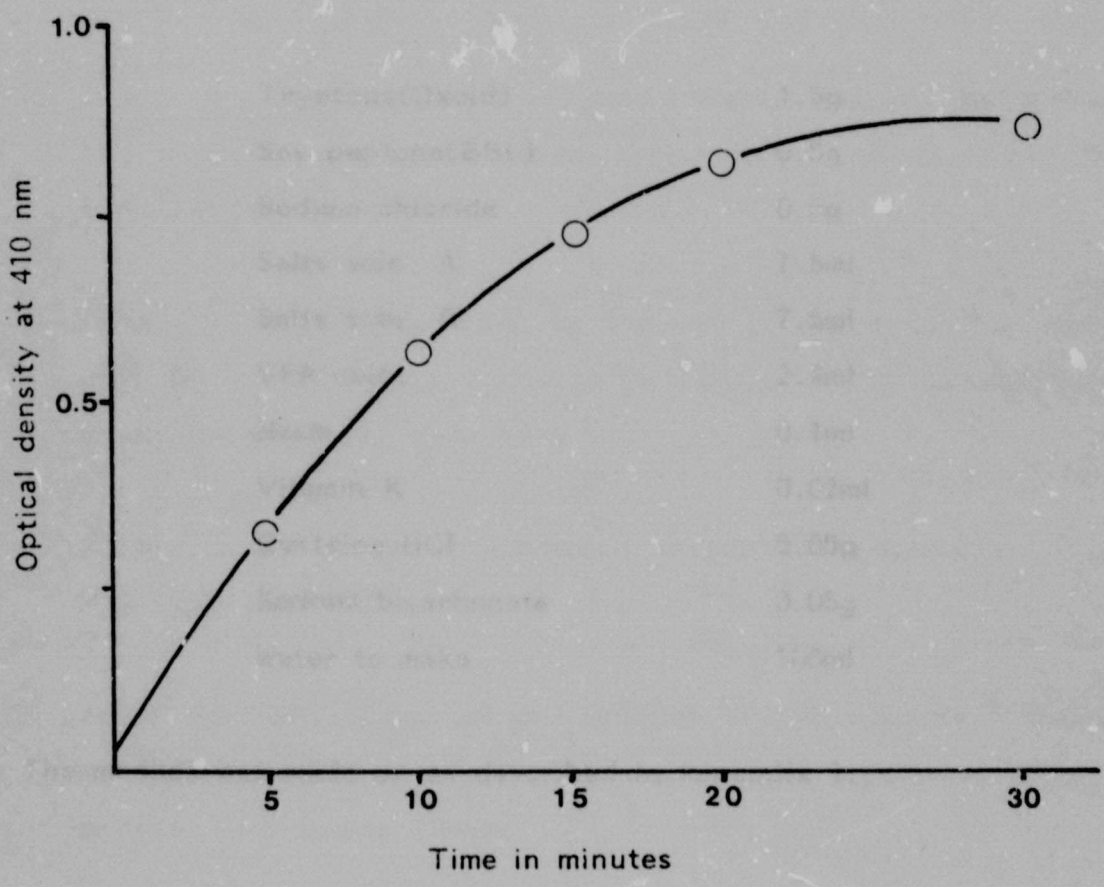

Figure 22. A_{410} of reaction mixtures in the standard enzyme assay plotted against incubation time in minutes.

Appendix 17. Tryptone soy broth

Tryptone(Oxoid)	1.5g
Soy peptone(BBL)	0.5g
Sodium chloride	0.5g
Salts soln. A	7.5ml
Salts soln. B	7.5ml
VFA soln.	2.4ml
Haem	0.1ml
Vitamin K	0.02ml
Cysteine HCl	0.05g
Sodium bicarbonate	0.05g
Water to make	100ml

The medium was made up as described in appendix 1.

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