Biochem. J. (1968), **108** 401
Printed in Great Britain

Composition of the Protoplast Membrane from *Saccharomyces cerevisiae*

By R. P. LONGLEY,* A. H. ROSE† and B. A. KNIGHTS
Department of Microbiology, University of Newcastle upon Tyne, and Department of Botany, University of Glasgow

(*Received* 28 *December* 1967)

1. Protoplasts of *Saccharomyces cerevisiae* N.C.Y.C. 366 were prepared by incubating washed exponential-phase cells in buffered mannitol (0·8M) containing 10mM-magnesium chloride and snail gut juice (about 8mg. of protein/ml. of reaction mixture). Protoplast membranes were obtained by bursting protoplasts in ice-cold phosphate buffer (pH 7·0) containing 10mM-magnesium chloride. 2. Protoplast membranes accounted for 13–20% of the dry weight of the yeast cell. They contained on a weight basis about 39% of lipid, 49% of protein, 6% of sterol (assayed spectrophotometrically) and traces of RNA and carbohydrate (glucan + mannan). 3. The principal fatty acids in membrane lipids were $C_{16:0}$, $C_{16:1}$ and $C_{18:1}$ acids. Whole cells contained a slightly greater proportion of $C_{16:0}$ and a somewhat smaller proportion of $C_{18:1}$ acids. Membrane and whole-cell lipids included monoglycerides, diglycerides, triglycerides, sterols, sterol esters, phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol + phosphatidylserine. Phosphorus analyses on phospholipid fractions from membranes and whole cells showed that membranes contained proportionately more phosphatidylethanolamine and phosphatidylinositol + phosphatidylserine than whole cells, which in turn were richer in phosphatidylcholine. Phospholipid fractions from membranes and whole cells had similar fatty acid compositions. 4. Membranes and whole cells contained two major and three minor sterol components. Gas–liquid chromatography, mass spectrometry and u.v. and i.r. spectra indicated that the major components were probably $\Delta^{5,7,22,24(28)}$-ergostatetraen-3β-ol and zymosterol. The minor sterol components in whole cells were probably episterol (or fecosterol), ergosterol and a C_{29} di-unsaturated sterol. 5. Defatted whole cells contained slightly more glutamate and ornithine and slightly less leucine and isoleucine than membranes. Otherwise, no major differences were detected in the amino acid compositions of defatted whole cells and membranes.

Although there are numerous reports on the lipid composition of micro-organisms (see Kates, 1964*a*, for review), relatively few studies have been made on the composition of isolated microbial membranes. Most of the studies reported have been on membranes from bacteria that can readily be converted into protoplasts (Salton, 1967). Among the protoplast membranes that have been analysed are those from *Bacillus megaterium* (Weibull & Bergström, 1958; Yudkin, 1966), *Bacillus subtilis* (Bishop, Rutberg & Samuelsson, 1967), *Micrococcus lysodeikticus* (Gilby, Few & McQuillen, 1958) and *Sarcina lutea* (Brown, 1961). A few studies have also been made on structures, probably corresponding to protoplast membranes, isolated from bacteria that are not readily converted into protoplasts. They include membranes from *Staphylococcus aureus* (Mitchell & Moyle, 1957) and *Streptococcus faecalis* (Ibbott & Abrams, 1964; Shockman, Kolb, Bakay, Conover & Toennies, 1963).

The only studies so far reported on the composition of protoplast membranes from yeasts are by Boulton (1965), who described briefly the gross composition of fractions obtained by centrifuging lysed preparations of *Saccharomyces cerevisiae* protoplasts at 1500*g* and 20000*g*, and Mendoza & Villanueva (1967), who reported a brief analysis of the composition of protoplast membranes of a

* Present address: School of Pharmacy, University of Wisconsin, Madison, Wis. 53706, U.S.A.

† Present address: School of Biological Sciences, Bath University.

strain of *Candida utilis*. The present paper reports a more detailed study on the composition of protoplast membranes from another strain of *S. cerevisiae*.

METHODS

Organism. The yeast used in this study was a strain of *S. cerevisiae*, N.C.Y.C. 366, which can readily be converted into protoplasts by using snail gut juice (Eddy & Rudin, 1958). It was maintained on slopes of malt wort–agar as described by Dixon & Rose (1964) and subcultured monthly.

Preparation and isolation of membranes. The yeast was grown in a glucose–salts–vitamins medium (Rose & Nickerson, 1956) supplemented with D-biotin (0·2mμg./ml.) and L-asparagine (0·2%, w/v). Supplementing the medium with asparagine increased the rate of growth of the yeast. Portions of medium were dispensed into flasks as described below, and were sterilized by autoclaving momentarily at 10lb./in.^{2}. Yeast from a freshly grown slope culture was suspended in a portion (10ml.) of medium in a Samco tube (Northam & Norris, 1951) to a concentration of 0·08–0·12mg. dry wt. equiv./ml. The culture was incubated statically at 25° for about 15hr., when the concentration of organisms was 0·37–0·44mg. dry wt. equiv./ml. A portion (4ml.) of this culture was added to medium (1l.) in a 2l. round, flat-bottomed flask, and the culture was incubated in a Perspex water bath through which was circulated water at 25°. The culture contained a follower magnet (4cm. long) and the contents of the flask were stirred at approx. 1250 revolutions of the follower magnet/min. After approx. 24hr. the whole culture was added to medium (3l.) in a 5l. round, flat-bottomed flask containing a 6cm. follower magnet, and the culture was incubated at 25° as already described for 6hr. The culture then contained 0·25–0·30mg. dry wt. equiv. of yeast/ml.

Yeast was harvested by centrifuging the culture in an International refrigerated centrifuge (model PR-2, no. 259 head), and the cells were washed three times with 67mM-KH_2PO_4, pH4·5, and once with water. A portion of the cell crop was freeze-dried and stored in a vacuum desiccator over silica gel at −20°. The remainder, which was used for preparing protoplast membranes, was suspended in 5mM-citrate–phosphate buffer, pH5·8 (Eddy, 1958), containing M-mannitol to a concentration of about 9·0mg. dry wt. equiv./ml. Ampoules (1ml.) of snail gut juice (the digestive juice of *Helix pomatia*) were pooled and diluted with an equal volume of water. The diluted juice was centrifuged at 20000*g* at 0° in an MSE Superspeed 40 ultracentrifuge (rotor no. 402). The supernatant liquid was removed and supplemented with 0·1vol. of 1% (w/v) cysteine hydrochloride (Sutton & Lampen, 1962) to inactivate the preservative added to the commercial preparation. One volume of diluted snail gut juice (containing approx. 40mg. of protein/ml.) was added to 4vol. of yeast suspension and shaken at 30° for 3hr. in a shaker–incubator (New Brunswick Scientific Co., New Brunswick, N.J., U.S.A.; Gyrotary, model G-25; 60rotations/min.). Examination of samples of suspension under a phase-contrast microscope showed that after 3hr. at 30° virtually all of the cells had been converted into protoplasts. Yeast protoplasts were separated by centrifuging the suspension for 10min. at 0° at 600*g* in an International refrigerated centrifuge. The protoplasts were washed twice at 0° with portions (100ml.) of citrate–phosphate buffer (pH5·8) containing 0·8M-mannitol and suspended in 100ml. of buffered mannitol (0·8M) containing 10mM-$MgCl_2$. The protoplast suspension was stored overnight at 4°.

To prepare protoplast membranes, the protoplast suspension was centrifuged for 10min. at 600*g* at 0° and the protoplasts were resuspended in a small volume of buffered mannitol (0·8M) containing 10mM-$MgCl_2$. The thick suspension was then squirted into 20vol. of ice-cold phosphate buffer, pH7·0 (Gomori, 1955), containing 10mM-$MgCl_2$, and the suspension was gently stirred at 0° for 30min. The suspension was then centrifuged at 1500*g* for 30min. at 0°. The supernatant liquid was removed and the membranes were resuspended by gentle stirring in phosphate buffer, pH7·0, at 0°, containing 10mM-$MgCl_2$, and again centrifuged at 1500*g* for 30min. at 0°. The membranes were washed once more in this buffer and then twice in water. Finally they were resuspended in a small volume (approx. 10ml.) of water in a stoppered tube and, unless used immediately, were frozen in liquid N_2 and freeze-dried. Membranes were stored in a vacuum desiccator over silica gel at −20°.

Extraction and analysis of lipids. Free lipids in cells and membranes were extracted by a modification of the method of Letters (1962). During this operation all solutions and suspensions were maintained under an atmosphere of N_2 gas. Freeze-dried cells (100–300mg.) or membranes (10–50mg.) were suspended in water (1ml.) in a 30ml. stoppered tube, and 9ml. of ethanol (99–100%, v/v) was added. The suspensions were flushed with N_2 gas, shaken and left for 24hr. at room temperature (18–22°) with occasional shaking. The suspension was then centrifuged, the supernatant liquid carefully removed with a pipette and the residue washed with ethanol (90%, v/v; 5ml.); the ethanol extracts were combined. The residue was suspended in 10ml. of chloroform–methanol (2:1, v/v) and left for a further 24hr. at room temperature. The suspension was then centrifuged and the supernatant liquid added to the ethanol extract, which had meanwhile been evaporated to dryness on a rotary evaporator (Buchler Instruments Inc., Fort Lee, N.J., U.S.A.) and dissolved in a small volume of chloroform–methanol. The combined extracts were evaporated to dryness, suspended in chloroform–methanol (12ml.) and washed by the method of Folch, Lees & Sloane-Stanley (1957) with water (3·0ml.). After separation of the two phases, the upper phase was removed and the lower-phase interface washed three times with chloroform–methanol–water (3:48:47, by vol.); the lower phase was evaporated to dryness. For determinations of free lipids, this material was weighed to constant weight. For analysis of the lipid, the material was redissolved in a small volume (1–2ml.) of chloroform.

Bound lipids remaining in the yeast after removal of free lipids were liberated by boiling the residue with 0·7N-HCl in methanol (4·5ml.) for 2hr. (Kates, Adams & Martin, 1963). Water (0·5ml.) was added to the suspension, which was then extracted with light petroleum (b.p. 40–50°; 3×5ml.) (Kates, 1964*b*). The extracts were evaporated to dryness under a stream of N_2 gas, dried and weighed.

Separation of lipids. Phospholipids and neutral lipids in the free-lipid extracts of membranes and whole cells were separated by thin-layer chromatography. Phospholipids were separated on plates (20cm.×20cm.) of silica gel (Whatman SG-41 or Camag silica gel without binder) by using a modification of the method of Skipski, Peterson &

Barclay (1964). After preparation, plates with layers 0·5mm. thick were allowed to dry for 1–2hr. on the leveller, and were then dried at 110° for 30min. They were stored in a desiccator until required. Immediately before use, the plates were activated by heating at 110° for 1hr., after which they were again placed in a desiccator to cool. Storage in a desiccator was necessary to ensure reproducible separations of phospholipids. The lipid extracts (1–50μl.) were applied with a micrometer syringe (Agla; A. Gallenkamp and Co. Ltd., London, E.C. 2) and the plates developed with chloroform–methanol–acetic acid–water (25:15:4:2, by vol.). The proportion of water in the mixture was decreased slightly on very humid days. This solvent carries the neutral lipids and phosphatidic acid with the solvent front. After the plates had been dried, the phospholipids were located by using a spray of Rhodamine 6-G (0·005%, in water) and by viewing under u.v. light (Hanovia Chromatolite). Rhodamine 6-G was always used to detect spots that were later subjected to fatty acid analysis. Phospholipids were also located with molybdenum blue reagent (Dittmer & Lester, 1964) and iodine vapour. Individual phospholipids were tentatively identified by using specific sprays and by comparing their R_F values with those of known phospholipids run on the same plate. Ninhydrin-positive phospholipids were identified by spraying plates with a solution of ninhydrin in butan-1-ol (0·2%, w/v) and warming the plates gently on an electric hot-plate. Choline-containing phospholipids were identified by spraying plates with the Dragendorff reagent (Skidmore & Entenman, 1962), and inositol- and glycerol-containing phospholipids by spraying with 0·1M-$AgNO_3$–aq. NH_3 soln. (sp.gr. 0·88) (1:1, v/v) followed by gentle warming on a hot-plate. In certain experiments, individual phospholipid spots were scraped off the plates, and the phospholipid was eluted from the silica gel with the developing solvent (2×3ml.), followed by methanol (2ml.) and methanol–acetic acid–water (94:1:5, by vol.; 2ml.; Skipski *et al.* 1964).

Neutral lipids were separated by thin-layer chromatography by a modification of the method of Wood, Imaichi, Knowles, Michaels & Kinsell (1964) with plates with layers of silica gel G 0·5mm. thick (E. Merck A.-G., Darmstadt, West Germany). The plates were developed with light petroleum (b.p. 40–60°)–diethyl ether–acetic acid (80:20:1, by vol.); with this solvent the phospholipids remained on the base line. Spots of neutral lipid were located with iodine vapour or with a spray of Rhodamine 6-G (0·005% in water) as already described. Individual neutral lipids were tentatively identified by comparing their mobilities with those of glycerol trioleate, oleic acid and ergosterol run on the same plates. For further studies, the spots were scraped off the plates and the lipids eluted from the silica gel with chloroform–methanol (2:1, v/v; 2×5ml.), followed by methanol (5ml.).

Extraction and estimation of sterols. The sterol contents of whole cells and membranes were determined by a modification of the method of Shaw & Jefferies (1953). A portion of freeze-dried whole cells (50–70mg.) or membrane (2–4mg.) was heated for 2hr. under reflux with KOH soln. (30–35%, w/v; 5ml.) at 115–120° on an oil bath in a 50ml. flask of the type described by Kates (1964*b*). After cooling, sterols were extracted with cyclohexane (2×10ml.). The extinction of this extract was measured at 271·5mμ, 282mμ, 293·5mμ and 310mμ with a cyclohexane blank, and the extinction readings were used to calculate the sterol content of the extract (as ergosterol) by using the equation given by Shaw & Jefferies (1953).

The contents of digitonin-precipitable sterols in whole cells were determined gravimetrically by a modification of the method of Sperry (1963). Portions (about 200mg. dry wt.) of cells were saponified with 40% (w/w) KOH soln.; the final concentration of KOH in the tubes was 30–35% (w/w). After cooling, the non-saponifiable materials were extracted with cyclohexane (3×10ml.) and the combined extracts made up to 50ml. Portions of the cyclohexane extract were then placed in tubes that had previously been weighed, and were evaporated to dryness under a stream of N_2 gas. The residue was dissolved in acetone–ethanol (1:1, v/v; 8ml.). Digitonin [2ml.; 2% (w/v) in 80% (v/v) ethanol] was added immediately, followed by water (2ml.), and the tubes were left overnight. The precipitate was washed with 80% (v/v) ethanol (2×3ml.), followed by diethyl ether (2×5ml.). For each washing, the precipitate was suspended with a glass rod, which was carefully washed with a few drops of solvent. After the final washing, the residual ether was blown off under a stream of N_2 gas. The tubes were then carefully wiped, and placed in an oven at 105° for 1hr.; after cooling in a desiccator, they were weighed.

Sterols that were to be separated by gas–liquid chromatography were also extracted as digitonides. After saponification with 40% (w/w) KOH soln., the material was extracted with diethyl ether (3×10ml.). The combined ether extracts were washed with water (2×10ml.), and filtered through glass wool. After being combined with the washings from the glass wool (3×5ml.), the extract was evaporated to dryness. The residue was then dissolved in 10ml. of acetone–ethanol (1:1, v/v). Digitonin [2% (w/v) in 80% (v/v) ethanol; 2·5ml.] was added to the solution, followed immediately by water (2·5ml.) (Sperry, 1963). The solution was left overnight at room temperature to ensure complete precipitation of the sterol digitonides. The precipitate was washed with portions (8ml.) of 80% (v/v) ethanol, acetone–diethyl ether (1:1, v/v) and finally anhydrous diethyl ether, and evaporated to dryness under a stream of N_2 gas. Free sterols were obtained from the digitonides by adding dry pyridine (0·6ml.) and heating the solution at 70° for 10min. After cooling, anhydrous diethyl ether (6·0ml.) was added, and the precipitate of digitonin was removed by centrifuging at 1500*g* for 10min. The supernatant solution containing the sterols was transferred to a 50ml. flask. The digitonin precipitate was washed with diethyl ether (3×5ml.), and the ether evaporated in a stream of N_2 gas. Pyridine was removed from the extract by placing the solution overnight in a vacuum desiccator over conc. H_2SO_4. The sterols were then dissolved in a small volume of light petroleum (b.p. 40–60°).

Gas–liquid chromatography. Methyl esters of fatty acids in lipid fractions were separated by gas–liquid chromatography with a Pye Panchromatograph (W. G. Pye and Co. Ltd., Cambridge) with a glass column (7ft.×0·4cm.) containing polyethylene glycol succinate (15%, w/w) on Chromosorb-W (60–80 mesh). The column temperature was in the range 160–170°, depending on the separation, and the rate of flow of the carrier gas (N_2) was 50ml./min. The apparatus was allowed to equilibrate for at least 24hr. before use. Methyl esters of the fatty acids were prepared by direct methanolysis, by the method of Stoffel, Chu & Ahrens (1959) modified by Kates (1964*b*). Portions (1μl.)

of extract were applied to the column with a microsyringe (Hamilton Co. Inc., Whittier, Calif., U.S.A.) through self-sealing septa. Methyl esters were detected with a flame-ionization detector (W. G. Pye and Co. Ltd.; catalogue no. 12210) with a mixture of H_2 (30ml./min.) and O_2 (100ml./min.). The analyser unit amplification was 1×10^{-8} or 3×10^{-8}. The signal from the detector was recorded on a continuous-balance potentiometer recorder (Honeywell Controls Ltd., Newhouse, Motherwell, Lanarkshire) with a chart speed of 15in./hr. Methyl esters of fatty acids were identified by comparing their retention times on the column with those of standard fatty acid methyl esters in mixtures. The compositions of these mixtures approximated to those designated A, B and D in the recommendations of Horning *et al.* (1964). A check on the linearity of the response of the detector was made by applying different amounts of mixture D (Horning *et al.* 1964) in 1μl. to the column and demonstrating that the apparent proportions of the different esters in this mixture did not vary at different detector amplifications. The areas of peaks corresponding to the separated esters were calculated by multiplying peak height by the width at half the height, measurements being made to the centre of the chart line. Peak areas were expressed as a percentage of the total area.

Trimethylsilyl ethers of sterols were prepared by evaporating the solution of sterols in light petroleum to dryness in a stream of N_2 gas, and dissolving the residue with gentle swirling in *NN*-dimethylformamide–hexamethyldisilazane–trimethylchlorosilane (40:40:1, by vol.) to a concentration of about 0·2mg. of sterol/ml. After standing at room temperature (18–22°) for 45min., the solution was evaporated to dryness under a stream of N_2 gas, and the trimethylsilyl ethers of the sterols were dissolved in a small volume of cyclohexane. The trimethylsilyl ethers were separated by using a Pye Argon chromatograph and columns (4, 7 or 9ft. long) of 1,4-dihydroxymethylcyclohexane succinate/polyvinylpyrrolidone (HI-EFF-8B/PVP) and a chlorophenylmethyl-type silicone (DC-560) on Gas-Chrom P (Knights, 1967*a*). Additional separations were done with a Pye 104 chromatograph (model 14) with a column (9ft. long) of silicone GE (SE-30; 1%) at 250°. Mass spectra of separated sterol trimethylsilyl ethers were obtained by using a gas chromatograph–mass spectrometer (LKB-9000) with 10ft. columns of SE-30. The molecular separator was operated at 250° and the ion source at 270°. The scan voltage was 70ev.

Amino acid analyses. Amino acids in defatted whole cells and in membranes were analysed with the Technicon amino acid auto-analyser. Portions of whole cells (500mg. dry wt.) or protoplast membrane (50mg. dry wt.) were suspended in water (5ml.) in a 30ml. stoppered test tube and heated in a bath of boiling water for 3min. The suspensions were then evaporated to dryness in a stream of N_2 gas. The residue was suspended in chloroform–methanol (2:1, v/v; 10ml.), and the suspension boiled gently by immersing the tube in a bath of boiling water before being evaporated to dryness in a stream of N_2 gas. The residue was suspended in 10ml. of chloroform–methanol (2:1, v/v) and, after cooling, centrifuged at 1500*g* for 10min. The supernatant liquid was removed and the extraction procedure repeated once more before the material was evaporated to dryness. Portions of the lipid-free material (1–5mg.) were weighed into narrow-necked hydrolysis tubes and 6N-HCl (5ml.) was added. After insertion of a plug of cotton wool to minimize entry of air, N_2 gas was blown gently on the surface of the liquid, care being taken to avoid splashing. The tubes were then sealed and placed in a bath of boiling water for 1hr. with occasional shaking. They were then placed in an oven at 110° for periods of 24, 48 and 72hr. After cooling, the tubes were broken into glass-stoppered test tubes and the solutions evaporated to dryness on a rotary evaporator to remove all traces of HCl. The hydrolysates were resuspended in sucrose solution (30%, w/v) containing 1mM-L-norleucine as an internal standard. The samples were then analysed for amino acids on a Technicon auto-analyser by using the buffer-gradient system of Thomson & Miles (1964).

Because of the extensive breakdown of cystine+cysteine and tryptophan during acid hydrolysis, the contents of each of these amino acids in whole cells and membranes were determined by other methods. The contents of cystine+cysteine (expressed as cysteic acid) were determined by the method of Moore (1963). A portion of whole cell or membrane (1–5mg. dry wt. equiv.) was suspended in 2ml. of performic acid [made by adding 1ml. of 30% (v/v) H_2O_2 to 9ml. of 88% (w/v) formic acid, allowing the mixture to stand at room temperature for 1hr. and cooling to 0°]. The suspension was left overnight at 0°. To each suspension 0·3ml. of 48% (w/v) HBr was added with swirling, and the mixtures were evaporated to dryness in a rotary evaporator, care being taken to remove all traces of HBr. Then 5ml. of 6N-HCl was added to the tube, which was sealed and heated at 110° as already described. The hydrolysates were suspended in 30% (w/v) sucrose soln. containing 1mM-L-norleucine and analysed in a Technicon auto-analyser.

Tryptophan was determined in alkaline hydrolysates of whole cells or membranes (Noltmann, Mahowald & Kuby, 1962). Samples (15–20mg. dry wt. equiv.) were suspended in water (0·5ml.) in Vitreosil digestion tubes; $Ba(OH)_2,8H_2O$ (6·3g.) and water (1·6ml.) were added to each tube; the tubes were then sealed in a stream of N_2 gas and heated at 110° for 70hr. Each tube was frozen and broken and the contents were transferred to a 100ml. plastic centrifuge tube with hot water. Then CO_2 was gently blown on to the surface of the suspension for 10min. with gentle shaking. After centrifugation for 15min. at 1500*g*, the supernatant liquid was removed and supplemented with water washings (2×5ml.). The solution was evaporated to approx. 1ml. in a rotary evaporator, filtered and freeze-dried. The hydrolysate was suspended in 30% (w/v) sucrose soln. containing 1mM-L-norleucine, and the samples were analysed in a Technicon auto-analyser.

Infrared spectra. Infrared spectra of trimethylsilyl ethers of sterols were obtained by using a Unicam SP.200G grating spectrophotometer. A solution of trimethylsilyl ethers was applied to NaCl plates that were gently heated with an i.r. lamp, and the spectra were determined by using the residual liquid film.

Analytical methods. Total nitrogen was estimated by the micro-Kjeldahl method (Markham, 1942) with 100-volume H_2O_2 as a catalyst (Miller & Miller, 1948). Total phosphorus was estimated by the method of Kolb, Weidner & Toennies (1963) after digestion of membranes with H_2SO_4 and H_2O_2 as a catalyst (Miller & Miller, 1948). Cold-acid extracts of membranes were prepared by extracting 2–3mg. dry wt. with two portions (4ml.) of 5% (w/v) $HClO_4$ at 0°. The extracts were combined and the extinction of the solution was measured at 260mμ in a Unicam SP.500 spectrophotometer (Schneider, 1945). The residue was resuspended in

1 ml. of water, and 4 ml. of 95% (v/v) ethanol was added to the suspension. The suspension was then centrifuged and the residue extracted again with 5 ml. of 95% (v/v) ethanol and then with ethanol (95%, v/v)–diethyl ether (3:1, v/v; 3 × 5 ml.); the extracts were combined. The residue was suspended in 4 ml. of 5% (w/v) $HClO_4$ and heated in a water bath at 90° for 15 min. to hydrolyse nucleic acids. After cooling, the suspension was centrifuged and the supernatant liquid, after being supplemented with washings (2 × 2 ml.) of 5% (w/v) $HClO_4$, was made up to 10 ml. with 5% (w/v) $HClO_4$. The extinction of this acid extract was then measured at 260 mμ and the extinction reading related to the apparent RNA content of the membrane by using the extinction coefficient 33·16 (de Deken-Grenson & de Deken, 1959). The residue was transferred to a micro-Kjeldahl flask and the total nitrogen content determined as described above. Total carbohydrate in cells and membranes was determined by the anthrone method (Wells & Dittmer, 1963). A modification of the H_2SO_4–carbazole (Ashwell, 1957) differential extinction method, described by McMurrough & Rose (1967), was used to determine the glucan and mannan contents of whole cells and membranes.

Chemicals. All chemicals used were A.R. grade or of the highest purity available commercially. Light petroleum (b.p. 40–60°) was redistilled before use. Cyclohexane was spectroscopic grade. D-Biotin was purchased from General Biochemicals Inc., Chagrin Falls, Ohio, U.S.A. Calcium D-pantothenate, pyridoxine hydrochloride and thiamine hydrochloride were supplied by Koch–Light Laboratories Inc., Colnbrook, Bucks. Other vitamins, amino acids, ergosterol, digitonin and *NN*-dimethylformamide were purchased from British Drug Houses Ltd., Poole, Dorset. Methyl esters of oleic acid, palmitoleic acid and stearic acid (99% pure), phosphatidylinositol, phosphatidylserine, trimethylchlorosilane and hexamethyldisilazane were obtained from Sigma (London) Chemical Co. Ltd., London, S.W. 6. Polyethylene glycol succinate, Gas-Chrom P, Chromosorb-W, HI-EFF-8B, PVP, DC-560 and SE-30 were supplied by Applied Science Laboratories Inc., State College, Pa., U.S.A. The digestive juice of *Helix pomatia* was supplied by L'Industrie Biologique Française S.A., Gennevilliers, Seine, France.

RESULTS

Overall composition

Portions of suspensions of washed yeast, and of suspensions of unwashed membranes obtained after protoplasts had burst, were filtered through weighed membrane filters (Millipore Filter Corp., Bedford, Mass., U.S.A.; pore size 0·45 μ; 2·5 cm. diam.), and the filters with the cells or membranes were washed and dried to constant weight at 90°. The dry weight of the protoplast membranes accounted for 13–20% of the dry weight of the cell.

Table 1 gives the overall composition of the protoplast membranes together with certain data on the composition of the whole cells. Most of the dry weight of the protoplast membrane was accounted for by protein and lipid. About 10% of the total lipid in whole cells was in a bound form. However, because of the small amounts of protoplast membrane available, it was not possible to analyse the membranes for bound lipid. Membranes contained larger amounts of lipid phosphorus and sterols (assayed spectrophotometrically) than whole cells. The sterol content of whole cells, when assayed gravimetrically, was greater than the value obtained with the spectrophotometric assay, which indicates that the cells contain compounds, presumably sterols, that combine with digitonin but have extinction properties that do not make them assayable by the method of Shaw & Jefferies (1953). Whole cells and membranes contained the

Table 1. *Overall composition of whole cells and protoplast membranes of* Saccharomyces cerevisiae *N.C.Y.C.* 366

Analytical procedures are described in the text. Carbohydrate contents of protoplast membranes are quoted as ranges. All other results are expressed as means ± S.D. of at least two determinations on each of two whole-cell and protoplast membrane preparations.

Component	Content (mg./100 mg. dry wt.)	
	Whole cells	Protoplast membranes
Lipid (free)	10·5 ±0·5	39·1 ±5·3
Lipid (bound)	1·2	—
Total N	7·6 ±0·6	9·1 ±1·1
RNA (by extinction)	7·0 ±0·1	7·0 ±0·6
Protein [(total N − RNA N) × 6·25]	40·5	49·3
Total carbohydrate	30·5 ±1·3	4·0 −6·0
Glucan	16·5 ±0·8	1·3 −2·6
Mannan	14·0 ±0·5	2·6 −3·6
Total phosphorus	2·15 ±0·02	1·21 ±0·07
Lipid phosphorus	0·101 ±0·005	0·25 ±0·03
Sterols		
Assayed spectrophotometrically as ergosterol	1·89 ±0·01	6·0 ±0·5
Assayed gravimetrically as digitonides	3·2 ±0·4	—

same proportion of u.v.-absorbing compounds (expressed as RNA).

Freeze-dried membrane, assayed directly for glucan and mannan by the sulphuric acid–carbazole method (McMurrough & Rose, 1967), gave an apparent mannan content of 14%; no glucan could be detected. However, the spectrum of the colour formed in this reaction had a maximum at 420mμ and a slight inflexion at 470mμ, which indicated that certain membrane components were probably interfering with the assay. When membrane constituents or related compounds were included in the assay mixture, it was shown that ergosterol interfered to the greatest extent, followed by phosphatidylserine; neither glycerol nor crystalline bovine plasma albumin caused any interference. Ergosterol reacting with sulphuric acid alone gave a solution with a maximum extinction at 420mμ, which suggests that interference was caused by a product of oxidation of ergosterol. Attempts were therefore made to assay the carbohydrate content of membranes after the saponifiable and non-saponifiable lipid materials had been extracted. Portions (3–10mg.) of freeze-dried whole cells or membranes were extracted with 3ml. of ethanol (95%, v/v)–diethyl ether (3:1, v/v;) in a bath of boiling water. After the supernatant liquid had been removed and the residue dried under a gentle stream of nitrogen gas, the residue was taken up in 1·5ml. of 35% (w/w) potassium hydroxide solution and heated on an oil bath at 115–120° for 2hr. After cooling, the non-saponifiable material was extracted with cyclohexane (2 × 10ml.), and traces of cyclohexane in the aqueous phase were removed under a stream of nitrogen gas. Water (4·85ml.) was then added so that the final concentration of potassium hydroxide was 13·3% (w/w). Portions

Table 2. *Fatty acid composition of the total free lipids of whole cells and protoplast membranes of* Saccharomyces cerevisiae *N.C.Y.C.* 366

Experimental details are given in the text. The fatty acids are designated $x:y$, where x is the number of carbon atoms and y the number of double bonds/molecule. Results are expressed as means ± S.D. of duplicate determinations on each of two samples of lipid from whole cells and membranes.

Fatty acid	% of total fatty acids	
	Whole cells	Protoplast membranes
10:0	0·3±0·1	0·1±0·1
12:0	1·0±0·2	0·4±0·1
14:0	1·9±0·2	1·5±0·2
15:0	0·7±0·2	0·5±0·1
16:0	13·7±0·4	11·4±0·1
16:1	47·8±0·5	48·6±1·0
18:0	6·0±0·3	6·3±0·3
18:1	28·6±0·5	31·3±1·0

Table 3. *Fatty acid composition of neutral-lipid fractions of whole cells and protoplast membranes of* Saccharomyces cerevisiae *N.C.Y.C.* 366

Experimental details are given in the text. The fatty acids are designated $x:y$, where x is the number of carbon atoms and y the number of double bonds/molecule. c, Whole cell; m, membrane. —, Amount of fatty acid present, less than 0·1%. Results are expressed as means ± S.D. of duplicate analyses on each of two samples of lipid from whole cells and membranes.

Fatty acid	% of total fatty acids in fraction									
	Monoglycerides + diglycerides		Free fatty acids		Triglycerides		Sterol esters		Phospholipids	
	c	m	c	m	c	m	c	m	c	m
10:0	0·5±0·2	—	—	0·1	0·3±0·1	—	0·8±0·2	—	—	—
12:0	1·1±0·2	—	1·2±0·3	0·2±0·1	0·7±0·2	0·1	1·7±0·7	1·0±0·1	0·2±0·1	—
14:0	1·8±0·2	0·6±0·2	2·7±0·2	1·3±0·2	2·6±0·2	0·9±0·2	2·7±0·4	3·0±0·3	0·9±0·2	0·5±0·2
15:0	0·8±0·1	—	—	0·4±0·2	0·9±0·1	0·3±0·1	1·0±0·2	—	0·3±0·1	—
16:0	12·3±1·2	6·3±1·6	13·8±1·3	10·0±0·7	16·0±1·1	8·4±0·9	7·2±2·7	16·4±2·2	13·9±1·3	12·0±1·0
16:1	49·1±1·5	49·2±1·5	45·5±2·5	51·9±1·1	42·3±1·4	49·8±1·2	58·7±4·0	46·5±3·0	45·1±1·3	42·7±1·1
18:0	6·7±0·7	2·8±0·9	9·3±0·8	6·0±0·7	10·0±0·7	6·2±0·8	4·8±2·1	9·9±1·3	4·9±0·9	5·0±0·7
18:1	27·7±1·0	41·1±1·4	27·5±1·5	30·1±0·8	27·2±0·7	34·3±0·9	23·1±1·6	23·2±1·4	34·7±1·5	39·8±1·1

of the suspension were placed in boiling tubes, and ethanol was added to bring the concentration of ethanol to 70% (v/v). The tubes were slowly warmed on a water bath until the contents just boiled, and were then left overnight at 4° to precipitate polysaccharides (Ghosh, Charalampous, Sison & Borer, 1960). After centrifugation, the supernatant liquid was removed and the residue evaporated to dryness under a stream of nitrogen gas. The residue was then resuspended in water (0·5ml.), and the glucan and mannan contents of the suspension were determined by the sulphuric acid–carbazole method (McMurrough & Rose, 1967). Analyses on whole cells showed that precipitating the polysaccharides by the procedure of Ghosh *et al.* (1960) caused an appreciable loss of polysaccharide (30–50%), the loss of glucan being greater than that of mannan. Values for the glucan and mannan contents of membranes (Table 1) were corrected for these losses.

Lipid composition

Total fatty acids. The total fatty acid compositions of whole cells and protoplast membranes were very similar (Table 2). The main differences were that the cells contained a slightly greater proportion of $C_{16:0}$ acids than the membranes, which in turn were somewhat richer in $C_{18:1}$ acids.

Neutral lipids. Thin-layer chromatography showed that the neutral-lipid compositions of the cells and the membranes were qualitatively almost identical. Monoglycerides, diglycerides, triglycerides, free sterols, sterol esters and hydrocarbons were detected in extracts of both materials. Judged from the intensities of the spots on the chromatograms after treatment with iodine vapour or spraying with Rhodamine 6-G, triglycerides and sterol esters were present in greater amounts than the other components.

The neutral lipids, and the phospholipids that remained on the base line when the neutral lipids were separated by thin-layer chromatography, had similar fatty acid compositions (Table 3). Hexadecenoic acids were the principal acids in each of the fractions, which also contained appreciable amounts of $C_{16:0}$ and $C_{18:1}$ acids. The main differences in the fatty acid compositions of the whole cells and the membranes were in the fractions containing monoglycerides + diglycerides; the membrane fraction had proportionately more $C_{18:1}$ acids in this fraction compared with the whole-cell lipids, which, in turn, contained slightly greater proportions of $C_{18:0}$ and $C_{16:0}$ acids.

Phospholipids. The phospholipid compositions of whole cells and membranes were also qualitatively similar. Both materials contained phosphatidylethanolamine, phosphatidylinositol + phosphatidylserine and phosphatidylcholine, with some lysophosphatidylcholine. Despite repeated attempts it was not possible to separate completely phosphatidylinositol and phosphatidylserine on thin-layer chromatograms; these phospholipids were therefore assayed together for phosphorus and fatty acids.

Phosphorus analyses of phospholipid fractions (Table 4) showed that membranes contained proportionately more phosphatidylinositol + phosphatidylserine than the cells, which in turn were slightly richer in lysophosphatidylcholine and phosphatidic acid. In general, the fatty acid compositions were similar for phospholipids from whole cells and protoplast membranes (Table 5). There were, however, certain differences, principally in phosphatidylethanolamine, which contained a greater proportion of hexadecenoic acids when extracted from whole cells than when extracted from protoplast membranes.

Sterol composition

Gas–liquid chromatography of the trimethylsilyl ethers of the digitonin-precipitable sterols from

Table 4. *Phosphorus contents of phospholipids from whole cells and protoplast membranes of* Saccharomyces cerevisiae *N.C.Y.C.* 366

Experimental details are given in the text. Results are expressed as means ± S.D. of four independent analyses of whole cells and protoplast membranes.

Phospholipid	Phosphorus content (% of total phosphorus recovered)	
	Whole cells	Protoplast membranes
Phosphatidylethanolamine	29 ± 1·5	33 ± 2·0
Phosphatidylinositol + phosphatidylserine	23 ± 2·0	28 ± 3·0
Phosphatidylcholine	27 ± 1·5	23 ± 2·0
Lysophosphatidylcholine	7 ± 2	2 ± 1
Phosphatidic acid	12 ± 2	11 ± 2
Material on base line	2 ± 1	3 ± 1

Table 5. *Fatty acid compositions of phospholipids from whole cells and protoplast membranes of* Saccharomyces cerevisiae *N.C.Y.C.* 366

Experimental details are described in the text. The fatty acids are designated $x:y$, where x is the number of carbon atoms and y the number of double bonds/molecule. PC, Phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid; NL, neutral lipid; c, whole cells; m, protoplast membrane. Results are expressed as means ± S.D. of duplicate analyses on each of two samples of lipid from whole cells and membranes.

	Fatty acid content of phospholipid (% of total in fraction, w/w)							
	PC		PI+PS		PE		PA+NL	
Fatty acid	c	m	c	m	c	m	c	m
10:0	—	—	—	—	—	—	0·3±0·1	—
12:0	0·1	—	0·1	0·1	0·1	—	1·2±0·1	0·4
14:0	0·3±0·1	0·3±0·1	0·3±0·1	0·2	0·4±0·1	0·8±0·3	2·4±0·2	1·3±0·2
15:0	0·1	—	0·2±0·1	0·1	—	—	0·8±0·1	0·4±0·1
16:0	5·7±2·1	5·1±1·7	26·2±1·6	23·2±1·4	5·8±2·2	13·0±1·5	12·8±0·6	9·3±0·5
16:1	57·9±2·8	53·1±2·5	23·4±1·4	24·2±1·1	54·3±1·6	42·6±2·9	50·2±1·1	51·2±0·9
18:0	1·0±0·5	2·0±0·7	14·5±0·7	14·2±0·8	0·7±0·2	2·2±0·6	7·2±0·4	6·0±0·3
18:1	34·9±1·4	39·5±2·1	35·3±1·8	38·1±1·9	38·7±1·2	41·4±2·7	25·1±0·9	31·4±0·6

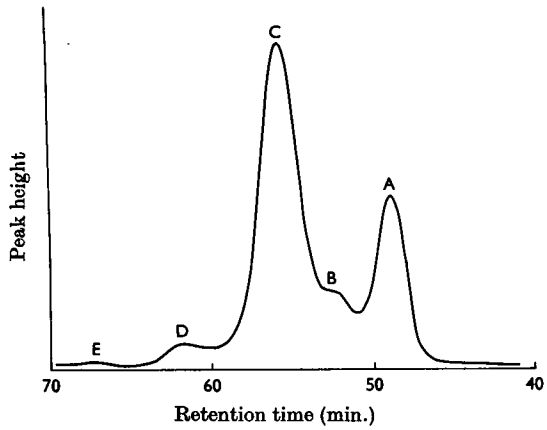

Fig. 1. Gas–liquid chromatogram of trimethylsilyl ethers of sterols from protoplast membranes of *Saccharomyces cerevisiae* N.C.Y.C. 366. Details of growth and harvesting of cells, preparation of protoplast membranes, and extraction and etherification of sterols are given in the text. The ethers were separated on a column of silicone GE (SE-30; 1%); the column temperature was 250°.

protoplast membranes (Fig. 1) showed two major (A and C) and three minor components (B, D and E). Very similar chromatograms were obtained when trimethylsilyl ethers of the digitonin-precipitable sterols from whole cells were separated. Components A and C from protoplast membranes and whole cells had almost identical mass spectra.

The trimethylsilyl ether of the major sterol component (C) had a relatively long retention time on HI-EFF-8B and a relatively short retention time on DC-560 (compared with ergosterol), which suggested that the side chain on the sterol might contain more than one double bond. The mass spectrum of the trimethylsilyl ether of component C (Fig. 2) showed a molecular ion of *m/e* 466, corresponding to a sterol of mol.wt. 394 and possibly therefore to a C_{28} tetraethenoid sterol. The peaks on the mass spectrum at *m/e* 335 and 131 (complementary ions resulting from a fracture through ring A of the sterol) suggested the presence of two double bonds in the sterol nucleus; this conclusion was supported by the presence of a peak at *m/e* 253 (equivalent to the molecular ion − 90 + side chain). The u.v. spectrum of the sterol digitonides showed the presence of the characteristic ergosterol $\Delta^{5,7}$-diene system with maxima at 271·5, 282, and 294 mμ, and a marked inflexion point around 263 mμ. The peak at *m/e* 251 on the mass spectrum (Fig. 2) suggested easy loss of the side chain from the sterol (molecular ion − 90 + side chain + 2H). An ion corresponding to molecular ion − 90 + side chain + 2H is a characteristic of sterols having a side-chain double bond (Knights, 1967*b*). Also, the relative intensity of the ion at *m/e* 251, and of the ion at *m/e* 123, which might have resulted from a charged side-chain fragment (C_9H_{15}), indicated the presence of two double bonds in the side chain. The strong absorption at 233 mμ was also indicative of a conjugated diene system other than the $\Delta^{5,7}$ system. The i.r. spectrum showed a peak at 970 cm.$^{-1}$ characteristic of a *trans*-disubstituted double bond (e.g. Δ^{22}) and a peak at 891 cm.$^{-1}$, which suggested the presence of a double-bond methylene group in the side chain of the sterol. These data were consistent with component C being $\Delta^{5,7,22,24(28)}$-ergostatetraen-3β-ol.

The sterol in component A in membranes and

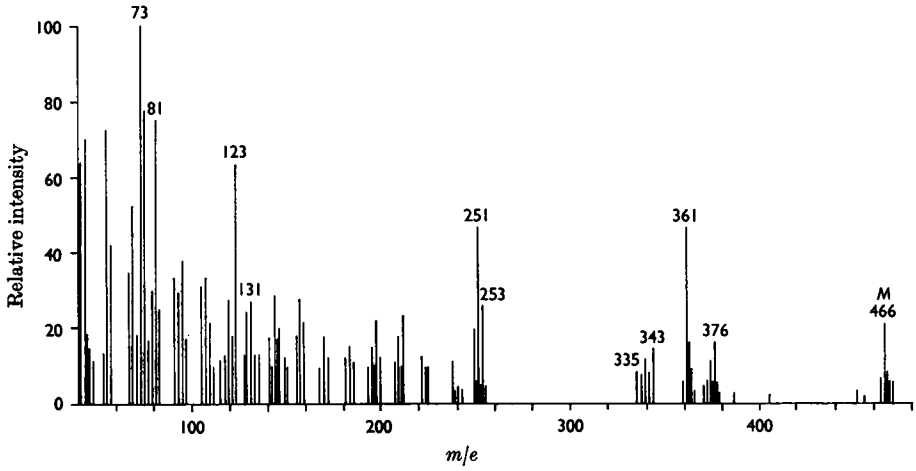

Fig. 2. Histogram of the more prominent peaks in the mass spectrum of the trimethylsilyl ether of the sterol in component C of the gas–liquid chromatogram (see Fig. 1). Only peaks with relative intensities greater than the following limits (relative to 100 for the peak at *m/e* 73) are shown: 10 in the range 40–250; 3 in the range 250–470.

whole cells was shown to be zymosterol on the basis of the similarity of its retention time (as the trimethylsilyl ether) on SE-30 and of its mass spectrum to those for authentic zymosterol (kindly determined by Dr C. J. W. Brooks).

Analysis of the minor sterol components (B, D and E) was done only on extracts from whole cells. The mass spectrum of component D showed a molecular ion at *m/e* 470 corresponding to a sterol of mol.wt. 398. The presence of peaks at *m/e* 386, 343 and 253 (involving respectively the loss of 84, 84 + 43, and 84 + 43 + 90 mass units) suggested that the sterol had a methylene group on C-24. The peak at *m/e* 213 was consistent with the presence of one double bond in the sterol nucleus. These data suggested that component D could be either $\Delta^{7,24(28)}$-ergostadien-3β-ol (episterol) (Knights, 1967*b*; Knights & Laurie, 1967) or $\Delta^{8,24(28)}$-ergostadien-3β-ol (fecosterol). The mass spectrum of component E was weak, but it showed a molecular ion at *m/e* 484, which was consistent with the sterol being a di-unsaturated C_{29} sterol with a mol.wt. of 412. A mass spectrum was not available for component B, but from its position on the gas–liquid chromatogram it was possibly ergosterol. Snail gut juice did not contain detectable amounts of sterols.

Amino acid compositions of defatted whole cells and of protoplast membranes

The amino acid compositions of defatted whole cells and of protoplast membranes were on the whole similar (Table 6). The main differences were in the contents of glutamic acid, isoleucine, leucine and ornithine, and these could be due to the fact that the amino acids in hydrolysates of defatted whole cells included those from the amino acid pool.

Table 6. *Amino acid compositions of whole cells and protoplast membranes of* Saccharomyces cerevisiae *N.C.Y.C.* 366

Hydrolyses of whole cells and protoplast membranes were carried out as described in the text. Hydrolyses were done for 24, 48 and 72 hr., and the amino acid contents quoted are maxima obtained by interpolation or extrapolation to zero time of these data. Results are expressed as means ± S.D. of two analyses of whole cells and protoplast membranes.

	Content (moles/100 moles of total amino acids)	
Amino acid	Whole cells	Protoplast membranes
Alanine	8·4 ± 0·4	7·7 ± 0·3
Aspartic acid	11·4 ± 0·1	10·8 ± 0·1
Arginine	4·8 ± 1·0	4·2 ± 0·2
Cysteic acid	1·5 ± 0·2	1·0 ± 0·1
Glutamic acid	12·8 ± 0·4	10·2 ± 0·2
Glycine	9·1 ± 0·2	8·3 ± 0·6
Histidine	2·2 ± 0·1	2·4 ± 0·2
Isoleucine	4·4 ± 0·4	5·5 ± 0·3
Leucine	7·2 ± 0·1	9·6 ± 0·3
Lysine	7·4 ± 0·3	7·4 ± 0·1
Methionine	1·4 ± 0·3	1·5 ± 0·2
Ornithine	1·9 ± 0·2	0·3 ± 0·1
Proline	3·9 ± 0·2	5·1 ± 1·0
Serine + threonine	10·8 ± 0·6	10·9 ± 0·3
Tryptophan	0·7 ± 0·1	1·2 ± 0·1
Tyrosine + phenylalanine	6·1 ± 0·3	7·8 ± 0·2
Valine	6·0 ± 0·6	6·1 ± 0·6

The amino acid composition of defatted whole cells was similar to that reported for another strain of *S. cerevisiae* by Jones, Power & Pierce (1965) and for *Nadsonia elongata* by Dyke (1964).

DISCUSSION

Before discussion of these data, it should be noted that the reagent used to digest the yeast cell walls, namely snail gut juice, contains several hydrolytic enzymes (Holden & Tracey, 1950). It is possible that, during the preparation of the protoplasts, some of these enzymes may have catalysed the hydrolysis of certain membrane constituents. Nevertheless, the extent of this hydrolysis was probably small, since yeast protoplasts prepared with snail gut juice have been reported to be physiologically active (Millbank, 1963).

The overall composition of the protoplast membranes from *S. cerevisiae* N.C.Y.C. 366 is very similar to the analyses reported for other microbial membranes (see the introduction for references), and in particular to the analyses of membrane-rich fractions from another strain of *S. cerevisiae* (Boulton, 1965) and protoplast membranes from *C. utilis* (Mendoza & Villanueva, 1967). Thus Boulton (1965) reported 38–45% of lipid and 46–47% of protein in the membranes of his strain of *S. cerevisiae*, and Mendoza & Villanueva (1967) found 40% of lipid and 38·5% of protein in the protoplast membrane of *C. utilis*. These values are similar to those reported in the present paper. The nitrogen and phosphorus contents obtained in the present analysis are also similar to those reported by Boulton (1965) from analysis of his 1.5p5 fraction. If it is assumed that an average of 4% of a phospholipid is phosphorus, then 15–20% of the lipid in the protoplast membranes analysed in the present study is phospholipid; the corresponding figure from Boulton's (1965) analysis was 25%. Our results for the apparent RNA and total sterol contents of the protoplast membrane also agree closely with those reported by Boulton (1965). Similar agreement is seen in the carbohydrate contents and compositions of the membranes. Mendoza & Villanueva (1967), on the other hand, reported that protoplast membranes from their strain of *C. utilis* contained 5·2% of carbohydrate, composed of galactose as well as glucose and mannose. The ranges obtained in our analyses of the glucan and mannan contents of the membrane carbohydrate make it difficult to decide whether this carbohydrate represents residual cell-wall material or a true membrane component, if indeed such a distinction can be made. Cell walls of *S. cerevisiae* contain approximately equal amounts of glucan and mannan (Mill, 1966), but the carbohydrate remaining on the protoplast membrane could be a mannan-rich fraction of the cell-wall polysaccharide.

Data on the phospholipid and fatty acid compositions of yeast protoplast membranes have not previously been reported. Phosphate analyses on phospholipids from whole cells and protoplast membranes of *S. cerevisiae* N.C.Y.C. 366 reveal certain differences, principally in the content of lysophosphatidylcholine, which is much greater in lipid extracted from whole cells than in membrane lipid. Harrison & Trevelyan (1963) noted the high content of this lysophosphatide in lipid extracted from baker's yeast, and attributed it to the action of a phospholipase A on phosphatidylcholine after the enzyme had been activated by ethanol used in extracting the lipid. As the protoplast membranes from *S. cerevisiae* N.C.Y.C. 366 contain proportionately less lysophosphatidylcholine than whole cells, it is possible that phospholipase A, if it is present in this yeast, is either inactivated or removed during preparation of protoplasts. Other differences between the phospholipid contents of whole cells and protoplast membranes could reflect differences in the phospholipid compositions of the outer cytoplasmic membrane and the intracellular membranes. During bursting of the protoplasts and subsequent washing of the protoplast membranes, it is likely that some intracellular membranes are washed away and, as the whole-cell lipid is relatively richer in phosphatidylcholine than the membrane lipid, it may be concluded that the intracellular membranes contain proportionately more phosphatidylcholine. Support for this suggestion comes from the report that there may be a relationship between the extent of intracytoplasmic membranes and the content of phosphatidylcholine in bacteria (Hagen & Goldfine, 1966).

A greater proportion of saturated fatty acid residues in the phosphatidylinositol + phosphatidylserine fraction than in other phospholipid fractions is found in lipid from both whole cells and membranes. A similar concentration of saturated fatty acid residues was reported by Trevelyan (1966) in phosphatidylinositol from baker's yeast, and by Ways & Hanahan (1964) in the phosphatidylinositol + phosphatidylserine fraction of human erythrocyte 'ghosts'. This preferential association of saturated fatty acid residues with phosphatidylinositol could be concerned with the physiological role of these phosphatides, conceivably by allowing the phospholipid molecules to be more closely packed in membranes (van Deenen, 1966). With the exception of the sterol ester fraction, the neutral lipids from protoplast membranes showed a slightly greater degree of unsaturation in their fatty acid residues compared with neutral lipids from whole cells. An explanation of this finding is not immediately apparent.

The overall similarity between the amino acid compositions of defatted whole cells and of protoplast membranes suggests that the specialized functions of membrane proteins, such as secretion and solute transport, do not confer on these proteins any peculiar amino acid composition. Robinson (1966) suggested that membrane proteins may be particularly rich in cysteine residues, and he provided data to support his contention. Our data do not lend support to this notion.

Sterols from protoplast membranes are chromatographically indistinguishable from those detected in whole cells; moreover, they are present in approximately the same proportions. The lack of agreement between values for the sterol content of whole cells assayed spectrophotometrically and gravimetrically appears to be due to the presence of zymosterol, episterol (or fecosterol) and the C_{29} di-unsaturated sterol, which are not assayed by the spectrophotometric method. Our data indicate that, in all probability, not all of the sterols in the cells are in the protoplast membrane. Assuming that the protoplast membrane accounts for 16% of the dry weight of the cell, and that 6% of the dry weight of the membrane is sterol (assayed spectrophotometrically), then the sterol content of whole cells should be about 1%, whereas the obtained figure is about 2%. It is conceivable that a certain proportion of the sterols in the cell is loosely associated with the membrane, and that this is lost during preparation of protoplast membranes.

The sterol composition of *S. cerevisiae* N.C.Y.C. 366 appears to be more complex than that of other strains of *S. cerevisiae*. Strain N.C.Y.C. 366 contains two major and three minor sterol components, whereas two other strains of *S. cerevisiae* and a strain of *C. utilis* (N.C.Y.C. 312) that were also examined contain only one major sterol, which is chromatographically identical with ergosterol (R. P. Longley & A. H. Rose, unpublished observations). The major sterol in *S. cerevisiae* N.C.Y.C. 366, namely $\Delta^{5,7,22,24(28)}$-ergostatetraen-3β-ol, has, however, been detected in baker's yeast by Breivik, Owades & Light (1954), though not as a major component. It is possible, too, that the strain of *S. cerevisiae* examined by Boulton (1965) contained this or a similar sterol, for the extinction of the sterol extract at 250 mμ was 40% greater than would be expected if ergosterol had been the sole major sterol present. Moreover, Katsuki & Bloch (1967) reported the production of this tetraethenoid sterol in reaction mixtures containing mevalonate, methionine and a cell-free extract of a strain of *S. cerevisiae*, as well as the conversion of the sterol into ergosterol by intact yeast cells. These workers claimed that the tetraethenoid sterol is a biosynthetic precursor of ergosterol in yeast, and it appears therefore that *S. cerevisiae* N.C.Y.C. 366, grown under the conditions used in the present study, has a restricted ability to convert the precursor into ergosterol. Nevertheless, the inability of this yeast to synthesize normal amounts of ergosterol does not apparently affect its ability to produce a functional protoplast membrane. The unusual sterol composition of the protoplast membrane in *S. cerevisiae* may conceivably be related to its having a cell wall that is more easily digested by snail gut juice than the walls of most other strains of *S. cerevisiae*.

We are extremely grateful to the Distillers Co. (Yeast) Ltd. for the award of a research studentship to R. P. L., and to Dr J. S. Harrison and Dr W. E. Trevelyan of the Distillers Co. for many valuable discussions. Our thanks are also due to Mr A. E. Thompson, of the Department of Organic Chemistry, University of Newcastle upon Tyne, for use of the amino acid auto-analyser. We are also grateful for the assistance given by Dr C. J. W. Brooks and Dr G. Eglington (co-directors), Dr A. McCormick (S.R.C. Research Fellow), and Miss H. Humphreys and Miss J. Malcolm in the Science Research Council gas chromatography–mass spectrometry unit, Department of Chemistry, University of Glasgow (supported by S.R.C. grant B/SR/2398). The Science Research Council under grant B/SR/2625 also generously provided funds for the purchase of a Pye Panchromatograph.

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