Fat Cell Plasma Membranes

I PREPARATION, CHARACTERIZATION, AND CHEMICAL COMPOSITION*

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Perirenal adipose tissue from rabbit, rat, and calf was disrupted without the use of proteases by a sieving procedure to yield fat cells from which plasma membranes were prepared. These membranes were isolated from the homogenate of the cells by differential and sucrose density gradient centrifugation. The major plasma membrane fraction, which represented the lightest band resolved from the microsomal pellet, was obtained from rabbit in a yield of about 4 mg/100 g of tissue and was purified about 17-fold in respect to 5'-nucleotidase activity. The membranes were rich in cholesterol and phospholipids (total lipid, 57% of membrane weight) and had high alkaline phosphatase activity. Only low levels of succinic dehydrogenase, NADPH-cytochrome c reductase, and nucleic acids were observed, indicating absence of significant contamination with intracellular components.

The amino acid compositions of the plasma membranes from the three species were quite similar, with glutamic acid, leucine, and aspartic acid occurring as the major constituents. The carbohydrate of the membranes, however, showed some species variation. The total saccharide content of the rabbit membrane was 8.7 mg/100 mg of protein and it was present as galactose, mannose, glucose, fucose, ribose, N-acetylneuraminic acid, glucosamine, and galactosamine in the molar ratios of 6.0:4.6:4.0:1.5:1.2:2.4:7.2:1.0, respectively.

An assessment of the protein and glycoprotein subunit composition of the rabbit, rat, and calf plasma membranes was made by polyacrylamide gel electrophoresis in sodium dodecyl sulfate with differential staining. While substantial species differences were apparent in the polypeptide pattern, the three membranes were alike in containing a major glycoprotein component (periodic acid-Schiff-reactive) with an apparent molecular weight in the range of 74,000 to 79,000. The rat membrane contained an additional prominent glycoprotein band with an apparent molecular weight of 88,000.

The most striking observation was the presence of the same major glycoprotein band(s) in all six membrane fractions obtained by density gradient centrifugation of the microsomal pellet. This finding may have a bearing on plasma membrane biogenesis in the fat cell.

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It has become apparent in recent years that a number of biologically active molecules including hormones, lectins, and toxins can influence the metabolism of the fat cell by interaction with components in its plasma membrane (1, 2). An understanding of these intermolecular events depends on a large measure on description of the chemical architecture of the cell surface, including the identification and structural analysis of the membrane components directly or indirectly involved. Indeed, the work of Cuatrecasas has already led to the solubilization and isolation of a specific insulin-binding protein from membranes of the adipocyte (3).

Methods hitherto reported for isolating plasma membranes from fat cells (4, 5) have utilized as the initial step the procedure of Rodbell (6) in which the adipose tissue is digested with crude collagenase. Since this enzyme preparation is known to contain a complex mixture of proteases (7, 8) and may also contain glycosidases (9) it would appear to be unsuitable for use in preparing membranes for analytical and structural investigations. This view is emphasized by reports that treatment of isolated fat cells with proteases or neuraminidase leads to cell surface changes which are translated into altered metabolic activities and hormonal responsiveness of the cell (10, 11).

Since it was the aim of the present study to undertake a detailed examination of the chemistry of the surface components of fat cells, a procedure for preparing plasma membranes was employed which, by relying entirely on mechanical disruption of the tissue, minimized the possibility of enzymatic degradation. The composition and enzymatic properties of membranes thus obtained from perirenal fat of rabbit, rat, and calf were determined and their protein and glycoprotein subunits were identified by polyacrylamide gel electrophoresis in sodium dodecyl sulfate.

EXPERIMENTAL PROCEDURES

Preparation of Plasma Membranes — Perirenal adipose tissue from fed young male rabbits (1.8 to 2.5 kg body weight) was obtained from Pel-Freez Biologicals and shipped to the laboratory in ice by air freight immediately after killing the animals. Since the tissue obtained in this manner yielded membranes which were indistinguishable from those prepared from rabbits killed in the laboratory, the commercial source was deemed to be satisfactory for securing the large amounts of material needed for our studies. Fresh perirenal adipose tissues were also obtained from fed female rats of the Charles River CD strain (260 to 280 g body weight) and from calves (approximately 1 month of age) slaughtered locally.

The adipose tissue after being dissected free of larger blood vessels was forced in 90-g portions first through a 42-mesh and then a 150-
mesh stainless steel sieve (8-inch diameter) with the bottom surface of a polycarbonate beaker. The passage through the sieves served to disrupt the tissue and yielded a paste representing, with weight, approximately 80% of the original tissue.

Portions (50 g) of the sieved fat were placed into 250-ml round-bottomed Erlenmeyer flasks and were digested with 5 ml of 0.25 M sucrose, 10 mM Tris/HCl buffer at pH 7.4 which was previously warmed to 32° for rabbit, 28° for rat, and 35° for calf tissue. The suspension was centrifuged at 220 x g for 2 min at room temperature in an International centrifuge to separate fat cells by flotation from a sediment of stromal cells. This pellet as well as the fluid underlying the fat cell layer were removed by aspiration. The layer of cells was washed by another centrifugation in the warmed buffered sucrose and was then suspended in 75 ml of the 0.25 M sucrose, 10 mM Tris/HCl, pH 7.4, buffer and disrupted with a single 5 s burst from a Polytron homogenizer equipped with a PT-20ST generator (Brinkmann Instruments) at a rheostat setting of 4. For this treatment the temperature of the medium was adjusted to 36° for rabbit, 32° for rat, and 38° for calf cells. The resultant homogenate was centrifuged at 750 x g for 2 min at room temperature to remove by flotation as yet unbroken fat cells as well as free fat, and subsequently chilled in an ice bath. The remaining fat cells were combined with buffer and rehomogenized as before by Polytron treatment to bring about their almost total disruption, and after removal of the fat layer the two homogenates were combined.

The homogenates from several 50-g portions of sieved fat were ultracentrifuged and fractionated by differential centrifugation at 2°. A nuclear pellet (N) was obtained by centrifugation at 800 x g for 15 min in an International refrigerated centrifuge. Successive centrifugations of the supernatants in a Beckman model L-5-65 ultracentrifuge at 13,000 x g for 15 min and 78,000 x g for 100 min yielded a pellet enriched in mitochondria (M) and a microsomal fraction (P), respectively, in addition to the final high speed supernatant (S). Three major protein fractions were washed twice by resuspension in approximately 50 ml of 0.35 M sucrose, 10 mM Tris/HCl, pH 7.4, buffer and centrifugation under the conditions initially employed for their sedimentation.

The pellet (P) was further fractionated by discontinuous sucrose density gradient centrifugation carried out in a Beckman SW-25.1 swinging bucket rotor for 14 h at 58,000 x g at 4°. The sucrose solutions employed in the gradient were prepared on the basis of a following solutions: 41% sucrose (1.42 M), 53% sucrose (1.65 M), 56% sucrose (1.71 M), 59% sucrose (1.75 M), 62% sucrose (1.78 M), 65% sucrose (1.82 M), 68% sucrose (1.86 M), 71% sucrose (1.90 M), 74% sucrose (1.95 M), 77% sucrose (2.00 M).

Enzyme Assays - 5'-Nucleotidase (EC 3.1.3.5) activity was measured by incubation of samples for 30 min at 37° in a total volume of 200 ml containing 10 µmol of Tris/HCl buffer (pH 8.0), 2.5 µmol of magnesium chloride, and 0.4 µmol of [14C]adenosine 5'-monophosphate (67 mCi/mmol). The reaction was stopped by heating 100 µl of the denatured protein by centrifugation, an aliquot of the supernatant (120 to 190 µl) was streaked on 3.7-cm strips of Whatman No. 1 paper and chromatographed in 95% ethanol:1 M ammonium acetate, pH 7.5 75:30, v/v for 22 h. In this chromatographic system AMP and adenosine were clearly separated (Rf of adenosine = 4.0) and the radioactivity in these components was localized and determined with a Nuclear Chicago radioscanner (Actigraph III) equipped with an integrator. Control incubations indicated that no significant amount of [14C]adenosine was formed in the absence of enzyme. The alkaline phosphatase (EC 3.1.3.1) was assayed for 15 min with p-nitrophenyl phosphate as substrate according to the method of Lansing et al. (12). Blank incubations were performed without enzyme.

Succinic dehydrogenase (EC 1.3.99.1) activity was determined essentially according to the procedure of Pennington with 2-(p-iodophenyl)-3-(p-nitrophenoxy)-5-phenyltetrazolium chloride as electron acceptor (13). Incubations were performed at 37° for 10 min in a total volume of 1.0 ml which contained the reagents at one-fifth of the concentration employed in the original procedure. Control incubation of all samples was performed in the absence of succinate. After subtraction of endogenous activity, enzyme activity was calculated from the molar extinction coefficient of the formazan product (13).

NADPH-cytochrome c reductase (EC 1.6.99.1) was assayed at 25° according to the procedure of Phillips and Langdon (14) by measuring the absorbance at 550 nm with a Gilford recording spectrophotometer.

All of the above enzyme assays were performed at protein concentrations within the linear range.

Chemical Analyses - Prior to the performance of chemical analyses the membranes were extensively dialyzed (96 h) against distilled water at 2° in the presence of a small amount of trisulfone, to remove sucrose and salts. Addition of tracer amounts of [14C]sucrose (New England Nuclear, 50 µCi/µmol) to the samples prior to dialysis permitted a monitoring of sucrose removal by the dialysis procedure.

For the determination of phospholipids and cholesterol the membranes were extracted with chloroform/methanol (2:1) by the procedure of Folch et al (15). The extracts were analyzed for phosphorous by the ascorbate method of Chen et al. (16) after digestion with 70% perchloric acid (17) and their phospholipid content calculated by multiplying the phosphorus value by a factor of 25. Cholesterol was determined by the procedure of Zlatkis et al. (18).

DNA was measured by the diphenylamine reaction of Burton (19) after hydrolysis of the samples in 0.5 M perchloric acid for 15 min at 90°.

For the determination of RNA the samples were hydrolyzed in 0.1 N KOH at 57° for 16 h followed by the addition of perchloric acid and a final concentration of 0.5 M at 20°. The RNA content of the supernatant was determined by the orcinol reaction for ribose (21) and correction for any hexose contribution to this reaction was made on the basis of measurements with the anthrone procedure (22) on separate aliquots of the alkali-treated samples.

Total phosphorus was determined by the ascorbate method (16) after digestion of the membranes with perchloric acid (17).

Individual neutral sugars were determined by borate complex anion exchange chromatography on a Technicon analyzer (23) after hydrolysis of samples in 1 N HCl for 5 h at 100° and passage of the hydrolysates through coupled columns of Dowex 50 (H+) and Dowex 1 (formate) (24).

Fucose was also determined on the unhydrolyzed samples by the procedure of Dacie and Shettle (24) with a 3-min heating period.

The monosaccharides were chromatographed on a Technicon system (25) after hydrolysis in 4 N HCl at 100° for 6 h and elution from a column of Dowex 50 (22), while glucosamine and galactosamine were resolved and separately determined on a Technicon NC-2 amino acid analyzer, with a program which utilized pH 5 buffers (25), after hydrolysis of the membranes for 15 h in constant boiling HCl in sealed tubes at 105°.

Sialic acid was released from the membrane by hydrolysis with 0.1 N H2SO4 at 80° for 1 h and was determined by the thiobarbituric acid assay of Warren (27) after 1% solutions on Dowex 1 (formate) columns (28).

Hexuronic acids were measured by the carbazole reaction of Dacie (28). In order to detect small amounts of these sugars without interference from hexoses, samples were hydrolyzed in 1 N HCl at 100° for 6 h. The hydrolysates were passed through coupled columns of Dowex 1 (formate) and Dowex 5 (OH-) and after washing with water, hexuronic acid was eluted with 0.5 N formic acid from the Dowex 1 column. The acid was removed from the eluate by lyophilization prior to assay by the carbazole reaction and paper chromatography.

Sulfate was determined by the benzenediazonium method of Dodson and Spencer (29) after hydrolysis of the samples in 4 N HCl at 100° for 22 h and removal of amino acids and peptides by passage through columns of Dowex 50 (H+) (30).

Amino acids were determined on a Technicon amino acid analyzer I (150 cm column) after hydrolysis of the samples in constant boiling HCl in sealed tubes at 105°. The values were not corrected for any destruction during the hydrolysis.

Protein was determined by the method of Lowry et al. (31) with bovine serum albumin as a standard. Membrane fractions were solubilized prior to the protein analysis by incubation with 0.1 N NaOH at 37° for 1 h.
Paper Chromatography—Chromatography of sugar components was carried out on Whatman No. 1 paper by the descending technique. Pyridine-ethanol-acetic acid (5:5:3:1) and pyridine-ethanol-water-acetic acid (11:4:6:5) in the bottom of the chromatography chamber (32) (System A) was employed for the identification of neutral sugars, hexosamines, and uronic acids, and 1-butyl acetate-acetic acid-water (3:2:1) (22) (System B) for sialic acids. The sugars were located on the chromatograms by the silver nitrate method (33, 34).

Phase Contrast and Electron Microscopy—A phase contrast microscope (Bausch and Lomb) was used for the examination of the fat cells. Electron microscopic examinations of membrane fractions were kindly performed by Dr. Arthur A. Like of the E. P. Joslin Research Laboratory. Membrane preparations from sucrose density gradients were diluted 15-fold with 0.25 M sucrose, 5 mM Tris/HC1, pH 7.4, and centrifuged at 100,000 x g for 80 min. The pellet was treated with a paraformaldeyde/glutaraldehyde mixture as the primary fixative and postfixed with osmium tetroxide. It was then imbedded in an Epon/Araldite mixture and thin sections were prepared with an LKB Ultratome which were stained sequentially with aqueous uranyl acetate and lead citrate (55). The stained sections were viewed with an RCA EMU-3G electron microscope.

Polyacrylamide Gel Electrophoresis in SDS—Electrophoresis was carried out in 7.5% polyacrylamide gels in glass tubes (0.5 x 12.5 cm) according to the procedure of Weber and Osborn (30) in a manner previously described (37). Prior to the electrophoresis the samples were incubated in 0.1 M sodium phosphate buffer, pH 7.0, containing 1% SDS and 1% 2-mercaptoethanol for 2 h at 37°C. The very small amount of insoluble material was removed by centrifugation at 1,000 x g for 20 min. Aliquots of the supernatants were applied to the gels and electrophoresis was performed with a current of 2 mA/tube for 16 h. The gels were stained with Coomassie blue for protein and with the periodic acid-Schiff procedure for carbohydrate according to the method of Farbanks et al. (38). The apparent molecular weights of the protein bands were determined from the migration of standard proteins, which included myosin, phosphorylase a, bovine serum albumin, ovalbumin, chymotrypsinogen, and myoglobin (36).

RESULTS

Isolation of Membranes from Rabbit Adipose Tissue—Examination under the phase microscope of the material obtained by flotation of the sieved adipose tissue indicated that it consisted of intact adipocytes, some broken fat cells and vesicles probably derived from the broken cell fragments. Connective tissue, blood vessels, and tissue fragments were entirely left on the sieves and stromal cells were found in the sediment obtained during centrifugation of the sieved tissue.

The Polytron homogenizer was effective in disrupting the fat cells, but the power setting of the instrument and the temperature of the medium substantially influenced the final yield and purity of the plasma membranes. The conditions of homogenization adopted included Polytron treatment at a low power setting for a brief interval in a medium sufficiently warm to soften the fat inside the cells. Different temperatures had to be selected for homogenization of fat cells from the three species studied, presumably reflecting differences in the fatty acid composition of the triglycerides.

Marker enzymes were assayed in the four subcellular fractions (N, M, P, and S) into which the homogenate was separated by differential centrifugation, 5'-Nucleotidase, which is believed to be a plasma membrane-bound enzyme, and NADPH-cytochrome c reductase, which is primarily a marker for endoplasmic reticulum, showed the highest specific activities in the P fraction, while the mitochondrial enzyme succinic dehydrogenase had the greatest specific activity in the M pellet.

Since the P pellet contained the largest amount of membrane-bound 5'-nucleotide activity, it was further submitted to a sucrose density gradient centrifugation which yielded six fractions (P, through P) (Table I). P was obtained as a distinct white band floating upon a light brown layer (P), which was present at the interface between layers of densities 1.032 and 1.142. P, and P, were faint bands which formed at the upper and lower interface, respectively, of the layer with a density of 1.161, while P, consisted of dark brown material which accumulated at the interface between layers with densities of 1.182 and 1.219, respectively. In addition, a small brownish pellet (P) was formed at the bottom of the centrifuge tube while some congealed free fat floated on the uppermost layer of the gradient.

Characterization of the fractions from sucrose density gradient centrifugation was performed by assays of marker enzymes and chemical components (Figs. 1 and 2). 5'-Nucleotidase activity was highest in the P, membranes but was low in the heavy fractions. Alkaline phosphatase, which is considered to be another enzyme associated with the plasma membrane, showed a similar distribution with most of the activity being localized in the lighter fractions from the density gradient. In contrast, succinic dehydrogenase and NADPH-cytochrome c reductase showed the highest specific activity in the heavy P, fraction. RNA, a marker for rough endoplasmic reticulum and free ribosomes was also recovered primarily in the heavy membranes (P, and P) while cholesterol, which is usually associated with plasma membranes, was concentrated in the light membrane bands (Fig. 2). The distribution of lipid phosphorus paralleled closely that of cholesterol so that this component was concentrated in the P, fraction.

On the basis of these analyses it became apparent that the P, fraction consists primarily of plasma membranes. P, represented 98% of the total protein in the P pellet from the rabbit adipose tissue (Table I) and had 17-fold greater 5'-nucleotidase activity than the unfractionated fat cell homogenate.

Electron microscopic examination of the P, fraction from

| Fraction | Protein (mg) | 5'-Nucleotidase (nmol/min/mg protein) |
|----------|-------------|--------------------------------------|
| Homogenate | 380 ± 47.2 | 3.7 ± 0.6 |
| Pellet—N | 0.9 ± 0.1 | 4.1 ± 0.5 |
| Pellet—M | 5.9 ± 0.8 | 9.5 ± 1.9 |
| Pellet—P | 15.8 ± 2.2 | 26.9 ± 2.3 |
| Supernatant—S | 322 ± 37.2 | 2.1 ± 0.3 |

Sucrose gradient membranes:

- P, (d: 1.080)'
  - P, (d: 1.130)'
  - P, (d: 1.154)'
  - P, (d: 1.170)'
  - P, (d: 1.197)'
  - P, (d: 1.233)'

The fractions are defined in Fig. 1.

The proteins values are for fractions derived from 100 g of the paste obtained by sieving of the adipose tissue. The recovery of protein from the homogenate in the fractions obtained by differential centrifugation was 91%, while 106% of the protein in the P pellet was recovered after sucrose density gradient centrifugation.

The recovery of the enzyme from the homogenate during differential centrifugation was 83% while 120% of enzyme activity in the P pellet was found in the sucrose density gradient fractions.

a The values in parentheses indicate the density of the fractions obtained by sucrose density gradient centrifugation.
rabbit adipose tissue indicated that it consisted primarily of membranous vesicles and membrane sheets. Flask-shaped invaginations which have been shown to be characteristic of fat cell plasma membranes (39) were observed in some portions of the larger vesicles. In some fields occasional ribosomes attached to membranes were noted, which could account for the low RNA content of the P, fraction. Components originating from mitochondria were very rarely seen.

Preparation of Membranes from Rat and Calf Adipose Tissue—For comparative purposes the procedure for isolating plasma membranes from the perirenal fat of rabbits was employed to prepare membranes from rat and calf. The procedure developed with rabbit tissue could be applied to these other two species without difficulty, and qualitatively similar patterns of membrane bands were obtained upon sucrose density gradient centrifugation of their P pellets. In rat and calf, as in rabbit, the P, fraction had the highest 5'-nucleotidase activity. This enzyme in P, was enriched over the homogenate 66, 79, and 86% respectively. The specific activities of the unfractionated P pellet in nanomoles/min/mg of protein for the other enzymes were: succinic dehydrogenase, 4.3; NADPH/cytochrome c reductase, 12.7; and alkaline phosphatase, 7.2. The recoveries of activity from the P, fraction were 66, 79, and 86% respectively. The designations of the fractions are described in text and Table I.

rabbit fat cell homogenates. Height of bars indicates specific activity of the enzyme in fraction, while width represents the percent of the total recovered protein. The values represent the average of three preparations. The recovery of protein from the P, pellet in the density gradient was 107%, while that of 5'-nucleotidase activity was 120% (Table I). The specific activities of the unfractionated P, pellet in nanomoles/min/mg of protein for the other enzymes were: succinic dehydrogenase, 4.3; NADPH/cytochrome c reductase, 12.7; and alkaline phosphatase, 7.2. The recoveries of activity from the P, pellet for these enzymes were 66, 79, and 86% respectively. The designations of the fractions are described in text and Table I.

![Fig. 1. Distribution of enzymes in membrane fractions obtained by sucrose density centrifugation of the P, fraction from rabbit fat cell homogenates.](http://www.jbc.org/)

![Fig. 2. Distribution of chemical components in membrane fractions obtained by sucrose density centrifugation of the P, fraction from rabbit fat cell homogenates.](http://www.jbc.org/)

### Table II

**Chemical composition of rabbit fat cell plasma membrane**

| Component       | mg/100 mg membrane protein |
|-----------------|-----------------------------|
| Protein         | 100                         |
| Phospholipid    | 196                         |
| Cholesterol     | 20.6                        |
| RNA             | 2.9                         |
| DNA             | 0.4                         |
| Carbohydrate    | 8.7                         |

a The membrane protein was determined from the amino acid analyses of the membrane.

b The membrane weight was calculated from the sum of the protein, lipid, nucleic acid, and carbohydrate analyses.

c Determined from the sum of the residue weights of the individual sugars in the membrane; the hexosamines were presumed to be present in the N-acetylated form.

### Table III

**Sugar composition of fat cell plasma membranes from three species**

| Species | Galactose | Mannose | Glucose | Fucose | Ribose | Glucosamine | Galactosamine | Sialic acid |
|---------|-----------|---------|---------|--------|--------|-------------|--------------|------------|
| Rabbit  | 10.2 ± 0.1 | 7.9 ± 0.3 | 6.8 ± 0.5 | 2.5 ± 0.5 | 2.1 ± 0.2 | 12.3 ± 0.4 | 1.7 ± 0.1 | 4.1 ± 0.3 |
| Rat     | 5.0       | 8.0     | 5.0     | 1.4     | 2.2     | 11.9        | 1.2          | 3.3        |
| Calf    | 4.7       | 4.6     | 4.7     | 2.0     | 2.0     | 12.6        | 2.0          | 3.2        |

a The membrane protein was calculated from the sum of the amino acid residue weights.

b Values for the rabbit membrane are given as the mean ± standard deviation of the mean of three preparations; the rat and calf analyses are for a single membrane preparation each.

c = galactosamine in the rat membrane was not determined but this sugar was identified by paper chromatography.

d Paper chromatography revealed that the sialic acid was present solely as N-acetylmuramic acid.

### Chemical Composition of Plasma Membranes—Analytical data for the chemical components of the plasma membrane fraction (P,) from rabbit are shown in Table II. The membranes contained a large amount of phospholipid, which was present in greater quantities than the cholesterol, so that the molar ratios of cholesterol to phospholipid was 0.33. The total phosphorus content of the P, membranes was 176 μmol/100 mg of protein, of which 163 μmol/100 mg (93%) was accounted for by the phospholipid. The remaining phosphorus of the membranes can largely be attributed to the small amounts of nucleic acids which are present.

The amino acid composition of the plasma membranes from rabbit as well as rat and calf adipose tissue was determined and found to be quite similar. The major components were glutamic acid, leucine, and aspartic acid, which were present as 97.8, 90.7, and 86.7 μmol/100 mg of membrane protein, respectively, in the rabbit. No hydroxyproline was detectable in the preparations, indicating the absence of basement membranes and collagens.

Ethanolamine, which originated from the acid hydrolysis of phosphatidylethanolamine, was measured on a Technicon amino acid analyzer where it emerged just prior to the ammonia peak and gave a molar color yield of 0.51 that of norleucine. From the value of this component (52.6 μmol/100 mg of
polyacrylamide gel electrophoresis in SDS of various fractions obtained from the sucrose density gradient of the P pellet was performed. Electrophoresis of the rabbit membranes after solubilization with 2-mercaptoethanol revealed, upon staining with Coomassie blue, that the light fractions (P1, P2, and P3) had a similar and relatively simple peptide pattern (Fig. 3). Five major components ranging in apparent molecular weight from 35,000 to 85,000 were evident in addition to several minor bands and some material which failed to penetrate the 7.5% gel. A considerably more complex electrophoretic pattern was observed in the heavier membrane fractions which are enriched in mitochondria and endoplasmic reticulum; in P5 and P6 particularly, a large number of bands in the low molecular weight range were seen.

Upon staining duplicate gels with the periodic acid-Schiff reagent all fractions contained one major discrete glycoprotein band which moved to a position corresponding to a molecular weight of 79,000 and appeared to coincide with a prominent Coomassie blue-positive component. In the light membrane fractions, P1 and P2, a broad band migrating just behind the tracking dye was observed after staining with the Schiff reagent (Fig. 3). This material which did not significantly react with Coomassie blue has been shown to be completely extractable with organic solvents (41) and probably represents cell-surface glycolipids which would be expected to be most prominent in the plasma membrane (P1) fraction.

When electrophoresis was performed on 5% gels rather than 7.5%, most of the material previously at the origin penetrated the gel slightly, consistent with molecular weights of greater than 250,000. Electrophoresis was, however, routinely performed in the 7.5% gels, as they gave the best separation of the components which were in the range of polyacrylamide gel resolution. No change in electrophoretic patterns was obtained when the membrane samples were heated in buffered SDS at 100° up to 30 min, nor when greater concentrations of reductant or SDS were employed.

An examination of the electrophoretograms from rat adipose
tissue membranes revealed a pattern distinct from that of the rabbit (Fig. 4). The light membrane fractions (P, through P3) when stained with Coomassie blue again presented only a few prominent bands. The two major Coomassie blue-reactive components had apparent molecular weights of 88,000 and 74,000 and corresponded in their migration to bands which stained with the periodic acid-Schiff reagent. These two glycoproteins appeared to be present in all the membrane fractions in a similar ratio to each other, although the fast migrating Schiff reagent-positive glycolipid material was essentially confined to P, and P2. As in the rabbit the heavier rat membrane fractions contained a large number of bands which stained with Coomassie blue and distributed over a wide range of molecular weights.

The electrophoretic patterns of the calf membranes were again different from the other two species (Fig. 5). Despite the presence of a large number of protein bands, particularly in the heavy fractions, only a single major glycoprotein band was observed which corresponded to an apparent molecular weight of 78,000.

The presence of the same major glycoprotein band(s) in all the membrane fractions obtained by density gradient centrifugation of the P pellet of a given species is a finding of some interest. Although the glycoprotein bands were most intense in the light plasma membrane fractions, their occurrence in the heavier membranes can not be attributed to cross-contamination in view of the enzymatic and chemical data (Figs. 1 and 2) and the observation that the periodic acid-Schiff-reactive glycolipid bands were not similarly distributed throughout the P fractions. Electrophoresis of the high speed supernatant (S) failed to reveal the presence of the same glycoprotein bands which were present in the membrane fractions, thereby excluding the possibility of nonselective binding of such cytosol components to the particles.

**DISCUSSION**

The present study has indicated that it is feasible to prepare plasma membranes from adipose tissue of several species without using a proteolytic digestion step. The procedure employed yielded a membrane preparation which on the basis of enzymatic, chemical, and morphological criteria appeared to be suitable for structural investigations. Components of the adipose tissue, other than fat cells, were removed by the sieving technique as well as by the flotation step. The plasma membranes were obtained in the lightest fraction after density gradient centrifugation of the microsomal pellet from the fat cells. They were primarily vesicular in nature and were characterized by high 5'-nucleotidase and alkaline phosphatase-specific activities as well as by a large cholesterol content. Polyacrylamide gel electrophoresis, moreover, indicated that glycolipids which are believed to be primarily cell surface components (42) were concentrated in this membrane fraction. The low to negligible levels of enzymatic and chemical markers for mitochondria, endoplasmic reticulum, nuclei, and basement membranes in this light membrane fraction further indicated that it was primarily of plasma membrane origin. Since the adipocyte is not endowed with an elaborate endoplasmic reticulum (49), it might be expected that the possibility of contamination with membranes of this network would be less than in more complex cells.

While the lightest membrane band (P,) obtained by density gradient centrifugation in this study was considered to be the primary plasma membrane fraction, it should be noted that the underlying band (P2) which contained substantially less cholesterol and phospholipid had, nevertheless, fairly high specific activities for the plasma membrane enzyme markers. Moreover, P, and P2 were essentially indistinguishable in terms of their polypeptide and glycoprotein components as seen by polyacrylamide gel electrophoresis and by amino acid and sugar analyses performed on the delipidated membranes (41). The occurrence of two plasma membrane fractions of distinct buoyant densities with different lipid contents has been previously observed in other cell types (44-46).

Although plasma membranes have been prepared by a number of investigators (4, 5, 47, 48) from rat adipose tissue enzymatically dissociated by the procedure of Rodbell (6), no detailed analyses of the peptide and carbohydrate constituents of these membranes have as yet been reported. The plasma membranes isolated by mechanical disruption of fat tissue in the present study were obtained in sufficient quantities from three species to permit complete amino acid and sugar determinations to be performed.

The fat cell membranes appear to be quite similar in their amino acid composition to the plasma membranes from a number of other sources (40, 44, 45, 49) in which glutamic acid, leucine, and aspartic acid are also the most abundant constituents. While the hexose, hexosamine, and sialic acid components of the fat cell membranes belong to glycoproteins and glycolipids (41), the small amount of ribose can readily be accounted for by the RNA present. The membranes from rat, rabbit, and calf adipose tissue had similar hexosamine compositions, but showed distinct differences in their neutral sugars analyses. The high ratio of glucosamine to galactosamine (approximately 7:1) in the adipocyte membranes is similar to that observed in the plasma membranes of human platelets (45) and calf thymocytes (50), but differs from that reported from human erythrocyte (50) and pig lymphocyte membranes (51) in which these two hexosamines are present in more equivalent amounts. The membranes from the latter cells have also been found to be relatively rich in citric acid and galactose.

Because of their high phospholipid content the rabbit fat cell plasma membranes have a molar ratio of cholesterol to lipid phosphorus (0.33) which falls into the lower portion of the rather wide range (0.24 to 1.3) which has been reported for this value in the surface membranes of various cells (52, 53). The overall analyses of the rabbit fat cell plasma membrane indicated that it had a high total lipid content compared to other plasma membranes (49) with a protein to lipid ratio of 0.67. The total carbohydrate contents of the fat cell membranes fell into the range (2 to 10% of membrane weight) reported for other plasma membranes from mammalian cells (49). The presence of small amounts of RNA as noted in the rabbit membranes from the present study has been observed in plasma membranes from various other sources (52), including those isolated from rat fat cells by the procedure of McKeel and Jarett (4).

Polyacrylamide gel electrophoresis of the membranes with differential staining permitted an assessment to be made of their protein and glycoprotein subunits. The plasma membrane fractions (P, as well as P2) of the fat cells from the three species examined had a rather simple pattern of polypeptide components when compared to the surface membranes of other cells (54) and to the heavier membrane of the fat cells themselves. Aside from the rapidly migrating glycolipid material, the periodic acid-Schiff reagent revealed only one major glycoprotein band in the rabbit and calf plasma membranes and two such components in the rat membranes, although additional
glycoproteins which either do not enter the polyacrylamide gel or fail to react sufficiently with the Schiff reagent may be present. The small number of visible glycoprotein components in the fat cell membranes contrasts with the situation which prevails in the membranes of liver (54, 55), kidney (54), and placenta (56) as well as fibroblasts (57), in which a large number of Schiff-positive bands have been observed. The human erythrocyte membrane is more similar to the fat membranes in demonstrating one predominant periodic acid-Schiff-reactive band upon gel electrophoresis (38, 54).

A comparison of the electrophoretic patterns of the plasma membrane fractions obtained from rabbit, rat, and calf in this study indicates that substantial species differences exist among the polypeptide components. The three species are alike, however, in containing a major Schiff-reactive glycoprotein subunit with an apparent molecular weight of 74,000 to 79,000. The rat membranes differed from the other two species in having an additional prominent glycoprotein band with a somewhat higher molecular weight (88,000). The occurrence of two such glycoprotein components has previously been observed in plasma membranes isolated from rat fat cells which were prepared by the enzymatic digestion procedure (47, 48). However, in the present study there was no evidence for a less prominent M, 62,000 glycoprotein band which has been observed in membranes from enzymatically dissociated rat fat cells (47). Furthermore, the larger number of Coomassie Blue-staining components, particularly in the lower molecular weight range, observed upon electrophoresis of plasma membranes prepared from enzymatically dissociated cells (47, 48) suggests the possibility that some proteolytic degradation of cell surface proteins may occur during preparation of fat cells by the collagenase digestion procedure.

One of the more striking observations made on the basis of gel electrophoresis was that in each species all of the membrane fractions from the microsomal (P) pellet contained the same major glycoprotein band(s). Since it was shown that this finding could not simply be the result of membrane cross-contaminations or of nonspecific adsorption of soluble glycoproteins, it would appear likely that it may have a significant bearing on plasma membrane biogenesis. Inasmuch as the relationship between the components of the endoplasmic reticulum and the cell surface membrane are not clearly understood, it will be important to characterize the glycoproteins which are found in these membranes.

The fat cell appears to be particularly attractive for the study of plasma membrane glycoproteins as it has a relatively simple complement of such molecules and furthermore is believed not to be involved in the synthesis of soluble glycoproteins for export.

In an accompanying report information in regard to the saccharide units of the plasma membrane glycoproteins from rabbit adipose tissue will be presented (41).

REFERENCES

1. Cuatrecasas, P., and Hollenberg, M. D. (1976) Ad. Protein Chem. 30, 251-451
2. Rodbell, M., Jones, A. B., DeCingolani, G. E. C., and Birnbauer, L. (1980) Recent Prog. Horm. Res. 24, 215-214
3. Cuatrecasas, P. (1972) J. Biol. Chem. 247, 1980-1991
4. McKeel, D. W., and Jarett, L. (1970) J. Cell Biol. 44, 417-432
5. Avruch, J., and Wallach, D. F. H. (1971) Biochim. Biophys. Acta 233, 334-347
6. Rodbell, M. (1964) J. Biol. Chem. 239, 375-380
7. Kono, T. (1968) Biochemistry 7, 1106-1114
8. Mitchell, W. M., and Harrington, W. F. (1968) J. Biol. Chem. 243, 4683-4692
9. Rosenthal, J. W., and Fain, J. N. (1971) J. Biol. Chem. 246, 5888-5895
10. Kono, T. (1969) J. Biol. Chem. 244, 1772-1778, 5777-5784
11. Cuatrecasas, P., and Illiano, G. (1971) J. Biol. Chem. 246, 4938-4946
12. Langerhans, A. L., Belkhode, M. L., Lynch, W. E., and Lieberman, I. (1967) J. Biol. Chem. 242, 1772-1775
13. Pennington, R. J. (1961) Biochem. J. 80, 649-654
14. Phillips, A. H., and Langdon, R. G. (1963) J. Biol. Chem. 237, 2652-2660
15. Faye, J. A., Loom, M., and Sarne, B. (1967) J. Biol. Chem. 242, 497-509
16. Chen, P. S., Jr., Toribara, T. Y., and Warner, H. (1965) Anal. Chem. 37, 1756-1758
17. King, E. J. (1932) Biochem. J. 26, 292-297
18. Zlatkis, A., Zak, B., and Boyle, A. J. (1953) J. Lab. Clin. Med. 41, 486-499
19. Burton, K. (1956) Biochem. J. 62, 315-323
20. Spiro, R. G., and Spier, M. J. (1966) J. Biol. Chem. 241, 1271-1282
21. Brown, A. H. (1946) Arch. Biochem. 11, 269-278
22. Spiro, R. G. (1966) Methods Enzymol. 8, 3-26
23. Spiro, R. G. (1972) Methods Enzymol. 28, 3-43
24. Dacie, Z., and Shattles, L. B. (1948) J. Biol. Chem. 175, 595-603
25. Boas, N. F. (1953) J. Biol. Chem. 204, 553-563
26. Spiro, R. G., Spier, M. J., and Bhnuyroo, V. D. (1976) J. Biol. Chem. 251, 6409-6419
27. Warren, L. (1959) J. Biol. Chem. 234, 1971-1975
28. Dacie, Z. (1947) J. Biol. Chem. 167, 180-190
29. Dodgson, K. S., and Spencer, B. (1953) Biochim. Biophys. Acta 55, 436-440
30. Spiro, R. G. (1967) J. Biol. Chem. 242, 1915-1922
31. Levery, O. H., Roobough, N. J., Parr, A. L., and Randel, B. J. (1951) J. Biol. Chem. 195, 265-275
32. Fischer, F. G., and Nebel, H. J. (1955) Hoppe-Seyler's Z. Physiol. Chem. 302, 10-10
33. Trevelyan, W. E., Proctor, D. P., and Harrison, J. S. (1956) Nature 166, 444-445
34. Benson, A. A., Basaham, J. A., Calvin, M., Hall, A. G., Illis, H. E., Kagawuch, S., Lynch, V., and Tolbert, N. E. (1952) J. Biol. Chem. 196, 703-716
35. Lico, A., and Glick, M. C. (1976) in Mammalian Cell Membranes (Jamieson, G. A., and Barber, A. J., and Jamieson, G. A. (1970) J. Biol. Chem. 245, 2130-2142
36. Barret, J. R. (1966) in Histology (Greep, R. O., ed) 2nd Ed, pp. 134-145 , McGraw-Hill Book Co., New York
37. Evans, W. H. (1970) Biochem. J. 166, 833-842
38. Barber, A. J., and Jamieson, G. A. (1970) J. Biol. Chem. 245, 6357-6365
39. Perdue, J. F., Kletzien, R., and Miller, K. (1971) Biochim. Biophys. Acta 249, 419-434
40. Czech, M. P., and Lynn, W. S. (1975) J. Biol. Chem. 248, 5081-5088
41. Avruch, J., and Lynn, W. S. (1975) J. Biol. Chem. 251, 1980-1981
42. Guidotti, G. (1972) Annu. Rev. Biochem. 41, 731-752
43. Kornfeld, R., and Siemers, C. (1974) J. Biol. Chem. 249, 2130-2138
44. Snary, D., Allen, A. K., Faulkes, R. A., and Crumpton, M. J. (1976) Biochem. J. 153, 75-78
45. Glick, M. C. (1976) in Mammalian Cell Membranes (Jamieson, G. A., and Robinson, D. M., eds) Vol. 1, pp. 45-77, Butterworths, London
46. Ashworth, L. A. E., and Green, C. (1966) Science 151, 210-211
47. Ginszmann, H., and Neville, D. M., Jr. (1971) J. Biol. Chem. 246, 6359-6364
48. Wray, M. H., and Evans, W. H. (1975) Biochem. J. 146, 375-388
49. Carlson, R. W., Wada, H. G., and Sussman, H. H. (1970) J. Biol. Chem. 521, 4139-4146
50. Wray, V. P., and Perdue, J. F. (1974) J. Biol. Chem. 249, 1189-1197
Fat cell plasma membranes. I. Preparation, characterization, and chemical composition.
Y Kawai and R G Spiro

J. Biol. Chem. 1977, 252:6229-6235.

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