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# COMPARISON OF PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM FRACTIONS OBTAINED FROM WHOLE WHITE ADIPOSE TISSUE AND ISOLATED ADIPOCYTES

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## SUMMARY

The influence of the mode of preparation upon some of the characteristics of white adipose tissue plasma membranes and microsomes has been reported. Plasma membrane fractions prepared from mitochondrial pellet were shown to have higher specific activities of $(Mg^{2+}+Na^{+}+K^{+})$-ATPase than plasma membranes originating in crude microsomes. Isolation of fat cells by collagenase treatment was found to result in a decrease in specific activity of the plasma membrane enzymes; in plasma membranes prepared from isolated fat cells, the specific activity values obtained for $(Mg^{2+}+Na^{+}+K^{+})$-ATPase and 5'-nucleotidase were only 42 % and 6.3 % respectively of those obtained in plasma membranes prepared from whole adipose tissue. Purification of whole adipose tissue crude microsomes by hypotonic treatment caused extensive solubilization of the endoplasmic reticulum marker enzymes, NADH oxidase and NADPH cytochrome *c* reductase. The lability of endoplasmic reticulum marker enzymes, however, was found to be greatly diminished in the preparations from isolated fat cells. The possibility that NADH oxidase and NADPH cytochrome *c* reductase activities found in the plasma membranes are microsomal enzymes adsorbed by the plasma membranes is discussed. The peptide patterns as well as the NADH oxidase and NADPH cytochrome *c* reductase activity patterns of plasma membranes and purified microsomes were compared by means of sodium dodecyl sulfate or Triton X-100 polyacrylamide gel electrophoresis.

## INTRODUCTION

The isolation of fat cell plasma membranes achieved by McKeel and Jarett [1] and Avruch and Wallach [2] was an important contribution to our knowledge of adipocyte subcellular organization. The fat cell plasma membrane has been found to contain specific enzymes: $(Mg^{2+}+Na^{+}+K^{+})$-ATPase [1–4], 5'-nucleotidase (EC 3.1.3.5) [2, 4–6] and hormone-sensitive adenylate cyclase [5]. This isolated plasma membrane has also been found to be the site of facilitated glucose transport [6, 7] as well as of specific receptors for hormones such as insulin [8] and glucagon [9].

The biochemical properties of white adipose tissue endoplasmic reticulum, however, are still not sufficiently well known. In only one study thus far, that of Avruch and Wallach [2], has any attempt been made to isolate a pure endoplasmic reticulum fraction from the crude fat cell microsomal pellet which is known to be contaminated with plasma membrane fragments [2, 4]. Glucose-6-phosphatase (EC 3.1.3.9), a typical endoplasmic reticulum marker enzyme in liver, could not be detected in white adipose tissue. Only one marker enzyme, in fact, NADH oxidase [1, 2, 4, 6], has been observed to characterize the endoplasmic reticulum membranes of adipose tissue. The specific activity of this enzyme, however, is generally found to exceed about three times only that of the plasma membranes, which suggests that there might be an NADH oxidase activity intrinsic to the plasma membranes [1, 2, 4]. The use of NADH oxidase as a marker enzyme for endoplasmic reticulum in adipose tissue might therefore be questioned.

The primary aim of the present study was to prepare the purest possible endoplasmic reticulum fraction and to distinguish this from the plasma membrane fraction by means of marker enzymes and gel electrophoresis. It was therefore necessary to verify the validity of using NADH oxidase as a marker enzyme for the endoplasmic reticulum fraction.

The second purpose was to compare the properties of plasma membrane and endoplasmic reticulum fractions prepared from isolated adipocytes with those prepared directly from whole adipose tissue. Previous studies of white adipose tissue have all used isolated adipocytes for the preparation of plasma membranes and microsomes. This preparation method, however, is not compatible with some types of studies of cellular metabolic compartmentalization, e.g. those in which in vivo administration of labeled precursors is followed by subcellular fractionation and analysis [10]. Since the metabolic processes in the living cell are extremely rapid and redistribution of the labeled molecules could take place during incubation with collagenase, any method involving a long incubation period should be avoided. Another disadvantage of fat cell isolation is that collagenase treatment might affect certain plasma membrane properties which could explain the modified hormonal responses observed in isolated fat cells [11, 12].

## METHODS

*Preparation of plasma membranes and purified microsomes*

Sprague-Dawley male rats, about 2 months old, weighing 200–250 g and fed Nafag chow (St.-Gall, Switzerland) ad libitum, were used. The rats were decapitated and the epididymal and perirenal white adipose tissues were excised and rapidly placed in 0.25 M sucrose, 10 mM Tris · HCl, 1 mM EDTA (pH 7.4) (sucrose/Tris/EDTA) at 4 °C. The tissues were cut with scissors into small pieces of about 2 $mm^2$. After homogenization plasma membranes and a crude microsomal fraction were isolated essentially as described by McKeel and Jarett [1] and the pure microsomes were isolated according to the technique described by Avruch and Wallach [2] but with the modifications described below. The isolation procedure was performed as follows: 20 g of tissue were homogenized in a Potter Elvejhem homogenizer at 1800 rev./min (Teflon pestle, 10 up-and-down strokes, clearance 0.3 mm) in 3 vol. of sucrose/Tris/EDTA which were then adjusted to 5 vol. The homogenate was

centrifuged at $1300 \times g$ for 20 min (MSE-18 centrifuge) and the supernatant was recentrifuged at $21\,000 \times g$ for 20 min. The resulting pellet (mitochondria+plasma membranes) was resuspended in 7.0 ml of sucrose/Tris/EDTA in a Potter Elvejhem homogenizer at 1500 rev./min (Teflon pestle, 6 up-and-down strokes, clearance 0.08 mm). The resuspended pellet was layered on 1 ml of sucrose density 1.14 containing 5 mM Tris at pH 7.4 and centrifuged at $83\,000 \times g$ for 30 min (Spinco L 50; rotor SW 39 L). The plasma membrane layer on top of the sucrose was shown by McKeel and Jarett [1] to contain only 10 % mitochondria. The plasma membranes were collected and diluted to 3.5 ml with 1 mM Tris · $MgSO_4$ at pH 8.6. The dilute plasma membrane solution was layered on 1 ml of sucrose density 1.13 containing 1 mM Tris · $MgSO_4$ at pH 8.6 and centrifuged at $134\,000 \times g$ for 35 min for repurification. The repurified plasma membrane layer on top of the sucrose was collected and recentrifuged at $175\,000 \times g$ for 30 min yielding the plasma membranes originating in the mitochondrial pellet and referred to as plasma membranes (mit). The crude microsome pellet was obtained by centrifugation of the $21\,000 \times g$ supernatant at $105\,000 \times g$ for 60 min and resuspended in 7.0 ml of 1 mM Tris · $MgSO_4$ at pH 8.6. The resuspended crude microsomes were layered on 1 ml of sucrose density 1.13 containing 1 mM Tris · $MgSO_4$ at pH 8.6 and centrifuged at $134\,000 \times g$ for 35 min. The plasma membrane layer on top of the sucrose and the 1 mM Tris · $MgSO_4$ at pH 8.6 supernatant were collected and recentrifuged at $175\,000 \times g$ for 30 min yielding the plasma membranes originating in the microsomes and referred to as plasma membranes (mc), and the hypotonic supernatant respectively. The pellet under the sucrose represented the purified microsomal fraction.

In some preparations, instead of whole adipose tissue, isolated fat cells, prepared according to Rodbell [13], were used. In the so-called paired experiments, one half of the rat epididymal and perirenal adipose tissue (left side for instance) was used for whole adipose tissue preparations, while the other half (right side) was used for isolated fat cell preparation. The isolated fat cells were homogenized either in sucrose/Tris/EDTA or in a medium containing 0.25 M sucrose, 5 mM $MgCl_2$, 25 mM KCl, 8 mM $CaCl_2$, 50 mM Tris · HCl at pH 7.5 [14] and referred to as $Ca^{2+}$ containing medium. The subcellular fractions were prepared as described for whole adipose tissue except that sucrose containing 1 mM Tris · $MgSO_4$ (pH 8.6) at a density of 1.14 instead of 1.13, was used when the isolated fat cells were homogenized in $Ca^{2+}$ containing medium. The pellets were resuspended in sucrose/Tris/EDTA and the proteins were measured according to Lowry et al. [15].

*Analytical methods*

The azide insensitive $(Mg^{2+}+Na^{+}+K^{+})$-ATPase activity was measured according to Herd et al. [16] in the presence of 20 mM azide. The use of this inhibitor of the $(Mg^{2+})$-ATPase allows measurements of an ATPase activity with an increased participation of the plasma membrane $(Na^{+}+K^{+})$-ATPase [3, 17]. 5'-nucleotidase was measured according to the method of Heppel and Hilmoe [18] and inorganic phosphorus by the method of Taussky and Shorr [19]. NADH oxidase was measured spectrophotometrically (Beckman DB-G Spectrophotometer) according to Strittmater [20] and NADPH cytochrome *c* reductase according to Sottocasa et al. [21]. Under the conditions of this study the enzymatic activities were found to increase linearly with the enzyme concentrations.

*Polyacrylamide gel electrophoresis*

Sodium dodecyl sulfate gel electrophoresis were performed essentially as described by Evans [22] except that the gels were 8 % in acrylamide and 0.4 % in bisacrylamide and that the large pore gel was omitted. Membrane pellets were allowed to dissolve at room temperature in a solution of 63 mM Tris · HCl buffer (pH 6.7), containing sodium dodecyl sulfate (3 %) and 10 mM mercaptoethanol. The membrane solution was then diluted twice with distilled water to a final protein concentration of about 0.5 mg of proteins per ml. In some cases, the membrane solution was heated at 70 °C for 20 min as described by Schnaitman [23], with no difference in the results. 0.15 ml of the solubilized membranes were applied to the gels and electrophoresis was run down with a current of 1 mA per gel for 6–7 h. Finally, the gels were stained with Coomassie blue according to Fairbanks et al. [24]. Molecular weight of the proteins on sodium dodecyl sulfate gels were estimated using thyroglobin, bovine serum albumin, ovalbumin, concanavalin A and cytochrome *c* as standards.

For determination of the ATPase activity on the sodium dodecyl sulfate gels, electrophoresis was performed as described above but with a current of 2 mA per gel. The gels were then washed 4 times with 50 ml of ice-cold distilled water and 4 times with 50 ml of ice-cold 80 mM Tris · HCl (pH 7.4) and incubated for 60 min at 37 °C in 10 ml of the ATPase medium [16]. At the end of the incubation period the gels were developed essentially according to the technique of Selwyn [25], i.e. 45 min incubation in 10 ml of 80 mM Tris-maleic acid buffer (pH 7.4), 3 mM $Pb(NO_3)_2$ at 37 °C followed by washing in distilled water and development in dil. $Na_2S$ solution. Blanks containing no ATP or proteins were also run.

For determination of the NADH oxidase and NADPH cytochrome *c* reductase on polyacrylamide gels, sodium dodecyl sulfate was replaced by 0.05 % Triton X-100 in the gel and 0.05 % in the electrophoresis buffer. The use of Triton X-100 was found to be necessary when preliminary electrophoresis in the presence of sodium dodecyl sulfate revealed no detectable NADH oxidase activity. Electrophoresis was performed at 4 °C with a current of 1.5 mA per gel for about 4 h. The upper buffer contained bromophenol blue as a tracking dye. The membrane pellets were dissolved and the NADH oxidase and the NADPH cytochrome *c* reductase were stained essentially as described by Skyring et al. [26] for bacterial dehydrogenase except that NADH (1 mM) or NADPH (0.5 mM) were used as substrates and that phenazine methosulfate was omitted from the developing solutions. Blanks without substrate or without proteins were run. In some NADH oxidase developing solutions, rotenone (1.5 $\mu$M) was added with no change in the gel patterns obtained. Densitometer tracings of the gels were obtained using a Gilford 2400 spectrophotometer at 560 nm.

## RESULTS

The plasma membrane and microsomal fractions were obtained either from whole adipose tissue homogenized in sucrose/Tris/EDTA, from isolated fat cells homogenized in sucrose/Tris/EDTA or from isolated fat cells homogenized in a medium containing $Ca^{2+}$. Table I shows the distribution of proteins among the plasma membranes isolated from the mitochondrial fraction (plasma membranes (mit)), the plasma membranes isolated from the microsomal fraction (plasma membranes (mc)) and the purified microsomal fraction obtained from 6 rats under the

TABLE I

QUANTITY OF PROTEINS FOUND IN PLASMA MEMBRANE AND PURIFIED MICROSOME FRACTIONS OBTAINED FROM 6 RATS

The results are the mean of the number of experiments indicated in parenthesis ±S.E. They represent in mg the total amount of proteins found in plasma membranes(mit), plasma membranes(mc) and purified microsomes obtained from the adipose tissue of 6 rats. The fractions were prepared either from whole adipose tissue or from isolated fat cells. A. Homogenization in sucrose/Tris/EDTA; B. Homogenization in $Ca^{2+}$-containing medium.

| | Total proteins | | |
|---|---|---|---|
| | Whole adipose tissue | Isolated fat cells (A) | Isolated fat cells (B) |
| Plasma membranes(mit) | 1.02±0.19(6) | 0.53±0.14(4) | – |
| Plasma membranes(mc) | 2.66±0.50(6) | 0.34±0.06(4) | 0.15±0.05(3) |
| Microsomes | 1.18±0.14(6) | 0.52±0.07(4) | 0.81±0.21(3) |

three experimental conditions described above. Of the fractions prepared from whole adipose tissue, the quantitatively most important is the plasma membrane (mc). The collagenase treatment decreased the yield of all three fractions studied but especially that of the plasma membranes (mc). When the isolated fat cells were homogenized in a $Ca^{2+}$-containing medium an effective separation of the plasma membranes (mit) was not achieved (see below) and the plasma membrane (mc) yield was very low.

Table II shows the results obtained when fractions were prepared from whole adipose tissue. The specific enzyme activities of plasma membranes (mit) and those of plasma membranes (mc) are compared with those of the purified microsomal fraction. The plasma membrane marker enzymes used were $(Mg^{2+}+Na^{+}+K^{+})$-ATPase and the 5′-nucleotidase. NADH oxidase was chosen as the marker enzyme for endoplasmic reticulum. The $(Mg^{2+}+Na^{+}+K^{+})$-ATPase and the 5′-nucleotidase spec. act. are 4.9 and 4.5 times higher respectively, in the plasma membranes (mit) than in the corresponding microsomes. The specific activities of the plasma membranes (mc) for both enzymes were intermediary between those of the plasma membranes (mit) and of the microsomes. Surprisingly, the specific activity of the microsomal marker enzyme NADH oxidase was 1.4 times higher in the plasma membranes (mit) than in the microsomes. When total activities are considered it can be seen that most of the $(Mg^{2+}+Na^{+}+K^{+})$-ATPase and 5′-nucleotidase activities are present in the plasma membranes while there is little remaining activity in the purified microsomes. This confirms the high degree of purification of the latter fraction.

The upper part of Table III shows the results obtained when the fractions were prepared from isolated fat cells homogenized under the same conditions as the whole adipose tissue. The $(Mg^{2+}+Na^{+}+K^{+})$-ATPase spec. act. was 5.2 times higher in the plasma membranes (mit) than in the corresponding microsomes indicating the same degree of purification of the plasma membranes (mit) as that achieved in the fractions separated from whole tissue. The specific activity of $(Mg^{2+}+Na^{+}+K^{+})$-ATPase in the plasma membranes (mc) was again found to be between that of the plasma membranes (mit) and that of the microsomes. In contrast with the results shown in Table II, the plasma membranes (mc) contribute only a small part of the total

TABLE II

COMPARISON OF PLASMA MEMBRANES AND PURIFIED MICROSOMES SEPARATED FROM WHOLE ADIPOSE TISSUE

The results represent the mean of the number of experiments indicated in parenthesis ±S.E. Specific activities (spec. act.) are expressed as μmol of $P_i$/mg proteins per min for $(Mg^{2+}+Na^{+}+K^{+})$-ATPase and 5′-nucleotidase, and as μmol of NADH oxidized/mg proteins per min for NADH oxidase. The corresponding total activities are expressed as μmol of $P_i$ formed or as μmol of NADH oxidized in 1 min in the total plasma membrane(mit), plasma membrane(mc) or microsome fractions obtained from adipose tissue of 6 rats.

| | $(Mg^{2+}+Na^{+}+K^{+})$-ATPase | | 5′-Nucleotidase | | NADH oxidase | |
|---|---|---|---|---|---|---|
| | Spec. act. | Total | Spec. act. | Total | Spec. act. | Total |
| Plasma membranes(mit) | 1.1 ±0.09 | 1.1 ±0.25 (6) | 0.58±0.04 | 0.65±0.06 (3) | 3.8±0.9 | 2.1±0.60 (3) |
| Plasma membranes(mc) | 0.80±0.07 | 2.0 ±0.39 (6) | 0.51±0.04 | 1.8 ±0.25 (3) | — | — |
| Microsomes | 0.22±0.02 | 0.30±0.06 (6) | 0.13±0.003 | 0.17±0.04 (3) | 2.8±0.6 | 2.7±0.38 (3) |
| Plasma membranes(mit) / microsomes | 4.9 ±0.6 | | 4.5 ±0.2 | | 1.4±0.1 | |
| Plasma membranes(mc) / microsomes | 3.6 ±0.3 | | 3.9 ±0.2 | | — | |

TABLE III

COMPARISON OF PLASMA MEMBRANES AND PURIFIED MICROSOMES SEPARATED FROM ISOLATED FAT CELLS

The results represent the mean of the number of experiments indicated in parenthesis ±S.E. Specific and total activities are expressed as in Table II. NADPH cytochrome *c* reductase spec. act. is expressed as nmol of NADPH oxidized/mg proteins per min.
(A) Homogenization in sucrose/Tris/EDTA; (B) Homogenization in $Ca^{2+}$-containing medium. The results for whole adipose tissue (Table II) and for isolated fat cells (A) are obtained from paired experiments (see Methods).

| | $(Mg^{2+}+Na^{+}+K^{+})$-ATPase | | 5′-Nucleotidase | | NADH oxidase | | NADPH cytochrome *c* reductase | |
|---|---|---|---|---|---|---|---|---|
| | Spec. act. | Total | Spec. act. | Total | Spec. act. | Total | Spec. act. | Total |
| (A) | | | | | | | | |
| Plasma membranes(mit) | 0.44 ±0.11 | 0.29 ±0.13 (3) | 0.036 | 0.014(2) | 3.8 ±0.9 | 1.8 ±0.38(3) | – | |
| Plasma membranes(mc) | 0.25 ±0.08 | 0.089±0.021(3) | – | | – | | – | |
| Microsomes | 0.084±0.028 | 0.048±0.025(3) | – | | 6.5 ±2.0 | 3.9 ±1.6 (3) | – | |
| Plasma membranes(mit) / microsomes | 5.2 ±2.2 | | – | | 0.58±0.06 | | | |
| Plasma membranes(mc) / microsomes | 3.0 ±0.8 | | – | | – | | – | |
| (B) | | | | | | | | |
| Plasma membranes(mc) | 0.19 | 0.038(2) | | | 1.8 ±0.3 | 0.25±0.05(3) | 0.18±0.05 | 0.027±0.008(3) |
| Microsomes | 0.041 | 0.051(2) | | | 5.5 ±2.9 | 3.3 ±0.62(3) | 0.72±0.31 | 0.56 ±0.12 (3) |
| Plasma membranes(mc) / microsomes | 4.6 | | – | | 0.33±0.14 | | 0.25±0.03 | |

$(Mg^{2+}+Na^{+}+K^{+})$-ATPase. The activities of plasma membrane enzymes were affected by collagenase treatment. The specific activities of $(Mg^{2+}+Na^{+}+K^{+})$-ATPase and 5'-nucleotidase were 2.4 and 16 times lower respectively, in fractions separated from isolated fat cells (Table III) as compared with those separated from whole adipose tissue (Table II). The lower part of Table III shows the results obtained when the fractions were prepared from isolated fat cells homogenized in a medium containing $Ca^{2+}$. As indicated by the spec. act. of $(Mg^{2+}+Na^{+}+K^{+})$-ATPase (not shown), an effective separation of plasma membranes (mit) from the mitochondrial pellet was not achieved. The $Ca^{2+}$ plasma membrane (mc) $(Mg^{2+}+Na^{+}+K^{+})$-ATPase activity, however, was comparable to that of sucrose/Tris/EDTA plasma membranes (mc).

The purification of the microsomes was improved when the fractions were prepared from isolated fat cells instead of whole adipose tissue. The lowest NADH oxidase plasma membranes/microsomes ratios were obtained when the fractions were prepared from isolated fat cells homogenized in the $Ca^{2+}$-containing medium (Table III).

NADPH cytochrome *c* reductase, a microsomal marker enzyme in liver [27] was also measured and gave similar plasma membranes/microsomes ratios as NADH oxidase. The specific activity of NADPH cytochrome *c* reductase in white adipose tissue was found to be about 100 times lower than that of the liver [27].

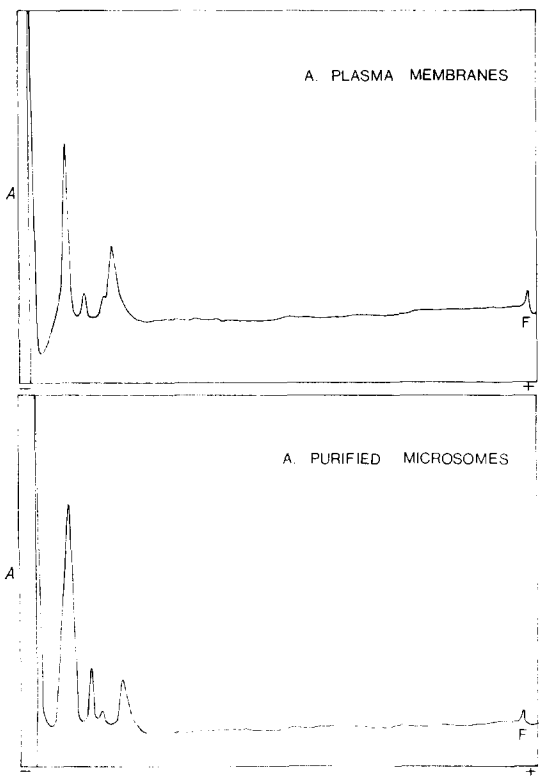
In paired experiments, the low specific and total NADH oxidase activities observed in microsomes prepared from whole adipose tissue (Table II) compared

TABLE IV

NADH OXIDASE AND NADPH CYTOCHROME *c* REDUCTASE SOLUBILIZATION, TOTAL ACTIVITIES

The results represent the mean of the number of experiments in parenthesis ±S.E. Total activities are expressed as μmol of NADH or as nmol of NADPH oxidized in 1 min in the total microsomes obtained from adipose tissue of 6 rats or in the total corresponding hypotonic supernatant (see Methods).
(A) Homogenization in sucrose/Tris/EDTA; (B) Homogenization in $Ca^{2+}$-containing medium. The results for whole adipose tissue and for isolated fat cells (A) were obtained from paired experiments (see Methods).

| | NADH oxidase | NADPH cytochrome *c* reductase |
|---|---|---|
| Whole adipose tissue | | |
| Microsomes | 2.7 ±0.4 (3) | 0.52 ±0.21 (3) |
| Hypotonic supernatant | 4.7 ±0.9 (3) | 0.71 ±0.20 (3) |
| Microsomes/Hypotonic supernatant | 0.57 ±0.06 | 0.73 ±0.31 |
| (A) Isolated fat cells | | |
| Microsomes | 3.9 ±1.6 (3) | – |
| Hypotonic supernatant | 0.36 ±0.04 (3) | – |
| Microsomes/Hypotonic supernatant | 10.8 ±3.9 | – |
| (B) Isolated fat cells | | |
| Microsomes | 3.3 ±0.6 (3) | 0.56 ±0.12 (3) |
| Hypotonic supernatant | 0.074±0.007 (3) | 0.042±0.006 (3) |
| Microsomes/Hypotonic supernatant | 44 ±9.2 | 13.3 ±1.3 |




with those observed in microsomes prepared from isolated fat cells (Table III) suggest that in the former preparation microsomal NADH oxidase might have been lost. This liberation of NADH oxidase might occur either in the supernatant of the 105 000 $\times g$ crude microsomal pellet or in the hypotonic supernatant (see Methods).

In Table IV, total NADH oxidase and NADPH cytochrome *c* reductase activities of the microsomal fraction are compared to those of the corresponding hypotonic supernatant in the three different preparations studied. When the fractions were prepared from whole adipose tissue, the total activity of both oxidases in the hypotonic supernatant was higher than that remaining in the microsomes. When the fractions were separated from isolated fat cells, however, the total oxidase activity found in the hypotonic supernatant was small as compared to that remaining in the microsomes. The total oxidase activity in the hypotonic supernatant might be considered to reflect oxidase lability. When the total NADH oxidase activity released in the hypotonic supernatant is the smallest (Table III: homogenization in $Ca^{2+}$-containing medium), a high activity is recovered in the microsomes whilst a low activity is found in the plasma membranes.

These results suggest that the oxidases which are presumed to be microsomal enzymes may, under certain conditions, leave the microsomes and be adsorbed by the plasma membranes. If this were true, the oxidases present in the microsomes and those found in the plasma membranes would have identical properties. This hypothesis is supported by the results shown in Fig. 1. It can be seen on polyacrylamide gels

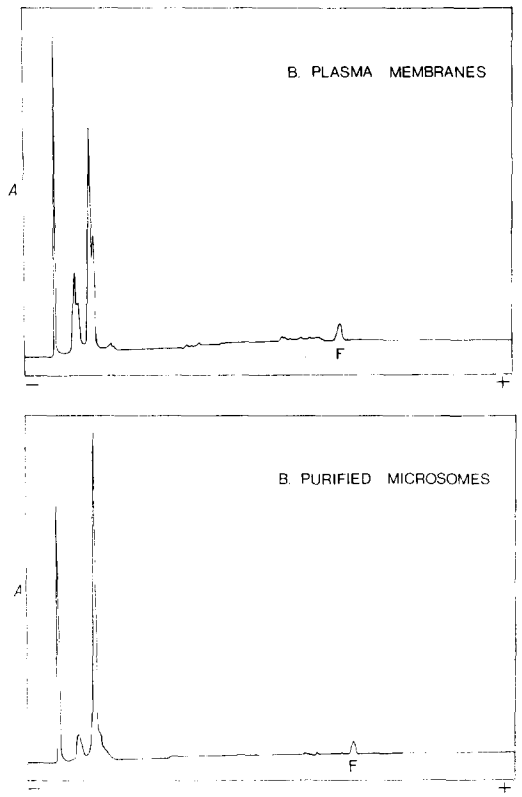



Fig. 1. Densitometer tracing of plasma membrane(mit) and purified microsome Triton X-100 polyacrylamide gels stained for NADH oxidase (A) and NADPH cytochrome *c* reductase (B). F = front. No bands could be detected in protein-free or NADH-NADPH substrate-free controls.

that NADH oxidase and NADPH cytochrome *c* reductase of both microsomes and plasma membranes (mit) separated from isolated fat cells and homogenized in sucrose/Tris/EDTA have comparable qualitative patterns. NADH oxidase is characterized by three main bands (Mean $E_f$ : $0.064 \pm 0.006$, $0.111 \pm 0.001$, $0.172 \pm 0.003$ for microsomes and 0.069, 0.111, 0.172 for plasma membranes (mit)). One part of the activity did not penetrate into the gel. NADPH cytochrome *c* reductase is characterized by one main band ($E_f$ : 0.125 for both microsomes and plasma membranes (mit)). A comparison of other enzyme properties would be necessary to confirm the identity of the plasma membrane (mit) and microsomal oxidases.

The protein pattern of the plasma membranes (mit) and that of the purified microsomes separated from isolated fat cells homogenized in sucrose/Tris/EDTA were compared (Fig. 2). Both fractions were composed of at least 15 major peptides on sodium dodecyl sulfate polyacrylamide gel electrophoresis. The majority of the protein peaks were common to both fractions. It may be noted, however, that peaks 2, 4 and 6 were virtually absent from the purified microsomes. Peak 9 which was present in the plasma membrane (mit) fraction was invariably predominant in the microsomes. No protein peak, however, under the present experimental conditions,

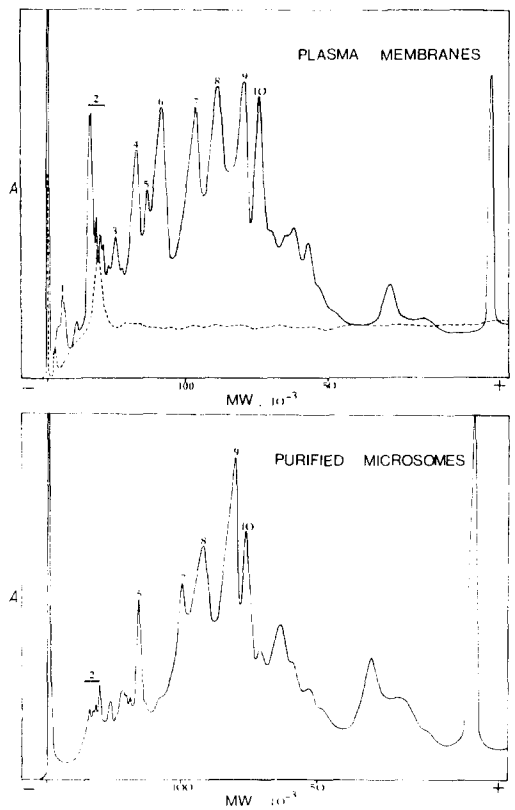



Fig. 2. Densitometer tracing of plasma membrane(mit) and purified microsome dodecyl sulfate polyacrylamide gels stained for proteins with Coomassie blue. The subcellular fractions were separated from isolated adipocytes and homogenized in sucrose/Tris/EDTA (see Methods). MW = molecular weight. · · ·, Polyacrylamide gel stained for ATPase. No band could be detected in protein-free or ATP substrate-free controls.

could be attributed exclusively to endoplasmic reticulum. The patterns shown in Fig. 2 were repeatedly obtained with at least four different preparations. The protein peaks present only in the plasma membranes were all of high molecular weight. Peak 2 is a group of five closely associated bands mol.wt $\times 10^{-3}$ ranging from $166 \pm 6.7$ to $155 \pm 2.1$. Peaks 4 and 6 have a mol. wt $\times 10^{-3}$ of $132 \pm 10.5$ and $115 \pm 5.2$ respectively. Among the plasma membrane marker enzymes $(Mg^{2+} + Na^{+} + K^{+})$-ATPase but not 5'-nucleotidase could be detected on the sodium dodecyl sulfate gels. $(Mg^{2+} + Na^{+} + K^{+})$-ATPase migrates with one of the plasma membrane peaks, i.e. peak 2 (mol. wt $\times 10^{-3}$ : 160). Peak 9, which is predominant in the endoplasmic reticulum protein pattern has a lower mol. wt ($\times 10^{-3}$) of $75 \pm 0.6$. The plasma membranes (mc) which are less pure than the plasma membranes (mit) with regard to marker enzyme activities have an intermediate profile between plasma membranes (mit) and microsomes on polyacrylamide gels. Peaks 2, 4 and 6 were smaller than in the plasma membrane (mit) pattern. Peak 9 was either the same as or greater than in the plasma membrane (mit) pattern (these results not shown).

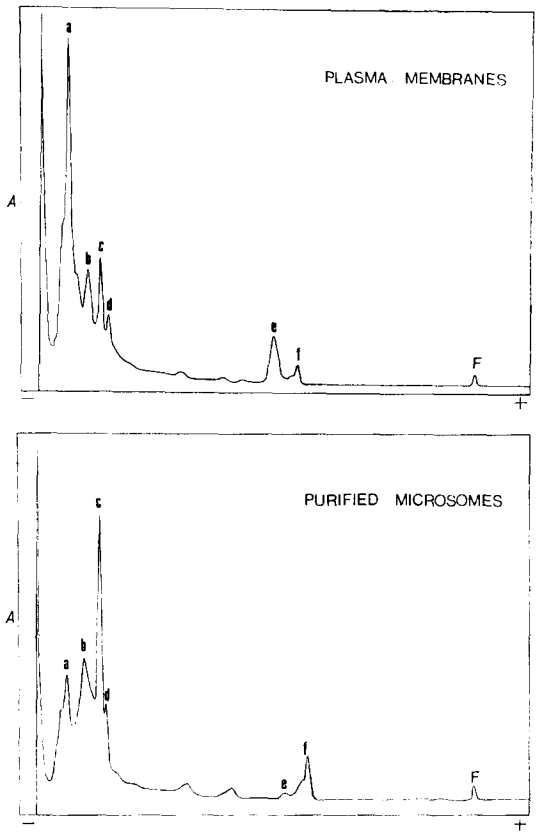



Fig. 3. Densitometer tracing of plasma membrane(mit) and purified microsome Triton X-100 polyacrylamide gels stained for proteins with Coomassie blue. The subcellular fractions were separated from isolated adipocytes and homogenized in sucrose/Tris/EDTA (see Methods). F = front.

Membranes prepared from isolated fat cells homogenized in a $Ca^{2+}$-containing medium could not be run on sodium dodecyl sulfate polyacrylamide gels since they were not satisfactorily dissolved by the solvent used in this study.

The protein pattern of the plasma membranes (mit) and that of purified microsomes separated from isolated fat cells homogenized in sucrose/Tris/EDTA on Triton X-100 polyacrylamide gels are compared in Fig. 3. It can be seen that a certain amount of proteins does not penetrate into the gel and that there are fewer bands than in sodium dodecyl sulfate polyacrylamide gels. Peaks a and e are predominant in the plasma membranes (mit) while peak c and possibly peak f are predominant in the purified microsomes. The first NADH oxidase band and the main NADPH cytochrome *c* reductase band (Fig. 1) seem to migrate with peaks a and c respectively of the Triton X-100 protein pattern.

## DISCUSSION

Although the use of plasma membranes or microsomes prepared from white

adipose tissue has been reported in numerous recent studies [3, 5–9, 11] many questions regarding these two important subcellular fractions remain unanswered. McKeel and Jarett [1] first described a technique for isolating white adipose tissue plasma membranes from the crude mitochondrial fraction while Avruch and Wallach [2] obtained plasma membranes from crude microsomes. A direct comparison, however, of these two different plasma membrane fractions has never been made. Moreover, plasma membrane fractions have always been prepared from isolated fat cells but those prepared from whole adipose tissue have not been studied. Finally, although NADH oxidase has been the only endoplasmic reticulum marker used, it has never proved really satisfactory in characterizing this fraction [1, 2, 4]. These problems have been the subject of the present study.

When plasma membrane fractions prepared from mitochondrial pellet (plasma membranes (mit)) were compared with those originating in microsomes (plasma membranes (mc)) it was found that the plasma membrane marker enzyme spec. act. were higher in the plasma membranes (mit) than in the plasma membranes (mc) (Tables II and III). It may thus be concluded that the large plasma membrane sheets sedimenting with the mitochondria are easier to isolate than the small plasma membrane vesicles sedimenting with the microsomes. The only exception was observed when isolated fat cells were homogenized in a medium containing $Ca^{2+}$. In this case, the degree of purification of plasma membranes (mit) was found to be very low while that of plasma membranes (mc) was normal. Of the fractions obtained from whole adipose tissue, the amount of proteins and of total $(Mg^{2+}+Na^{+}+K^{+})$-ATPase activities was higher in plasma membranes (mc) than in plasma membranes (mit), while in those obtained from isolated cells, the reverse is true. The presence of $Ca^{2+}$ in the homogenizing medium resulted in a pronounced decrease in the yield of the plasma membranes (mc). This might possibly be due to the fact that the majority of the plasma membrane fragments have sedimented with mitochondria. However, the technique used in the present study did not allow for their separation from mitochondrial fraction. The above results seem to indicate a correlation between the conditions of homogenization and the yield of large rather than small plasma membrane vesicles.

When plasma membrane and endoplasmic reticulum fractions prepared from whole adipose tissue were compared with those prepared from isolated fat cells, the following observations were made:

The collagenase treatment decreases the spec. act. of the plasma membrane enzymes $(Mg^{2+}+Na^{+}+K^{+})$-ATPase and 5′-nucleotidase, the latter in particular (Tables II and III). This is compatible with the fact that in the plasma membranes of white adipose tissue little or no 5′-nucleotidase activity could be detected by Avruch and Wallach [2] and McKeel and Jarett [1], respectively. Thus either the 45 min incubation at 37 °C or the action of the proteolytic enzymes contaminating the collagenase preparation [11, 12], or both, considerably affect the two plasma membrane enzymes.

The marker enzymes of endoplasmic reticulum fragments prepared from isolated fat cells have higher specific and total activities than those of endoplasmic reticulum fragments prepared from whole adipose tissue. This could be explained by the fact that in the latter case, the endoplasmic reticulum marker enzymes i.e. NADH oxidase and NADPH cytochrome *c* reductase are solubilized. Moreover, the correlation between NADH oxidase solubilization and its activity in the plasma membranes

(Tables II–IV) as well as gel patterns of the endoplasmic reticulum marker enzymes (Fig. 1) suggest that the solubilized microsomal enzyme might be adsorbed by plasma membrane fragments which would further complicate interpretation of the results. The different lability of whole adipose tissue and isolated fat cell microsomal enzymes might be due to the different conditions of homogenization. The pressure upon the cells during homogenization is probably greater for whole adipose tissue which might induce lability of the microsomal enzymes by modifying the microsomal structure. This lability could explain the difficulties encountered in testing the purity of white adipose tissue microsomes when NADH oxidase is used as a marker [1, 2, 4]. Since lability of some microsomal enzymes has also been observed in liver [10, 28, 29], it is possible that loose binding of enzymes with membrane structure is generally characteristic of endoplasmic reticulum.

It should be born in mind that cellular fractions prepared from whole adipose tissue probably contain, in addition to adipocytic membranes, fragments originating in the stroma-vascular tissue. It is unlikely, however, that this extra-adipocytic contamination is the cause of the high $(Mg^{2+}+Na^{+}+K^{+})$-ATPase and 5′-nucleotidase activities observed in the whole adipose tissue plasma membranes or of the presence of NADH oxidase in the whole adipose tissue hypotonic supernatant. Membrane fragments separated after collagenase treatment from the stroma-vascular cells of adipose tissue by the same technique as that used for plasma membranes (mit) and purified microsomes were not, in fact, found to have high $(Mg^{2+}+Na^{+}+K^{+})$-ATPase or 5′-nucleotidase activities nor was NADH oxidase found in the hypotonic supernatant (results not shown).

In the present study, the gel patterns of plasma membranes (mit) and of a purified microsomal fraction were compared. The results showed an enrichment of the plasma membrane fractions in high molecular weight peptides and the virtual absence of these peptides in the microsomes. Czech and Lynn [11], comparing the gel patterns of plasma membrane and crude microsomal fractions prepared according to the method of McKeel and Jarett [1], obtained substantially the same results. The differences in the molecular weights observed by Czech and Lynn [11] compared with those found in the present study might be due to their use of urea in the membrane solubilizing solution. The 166 000–155 000, 132 000 and 115 000 bands were invariably observed in the plasma membrane (mit) fraction suggesting that they could be used as criteria for identifying white adipose tissue plasma membranes. It is noteworthy that the $(Mg^{2+}+Na^{+}+K^{+})$-ATPase activity was found to migrate with one of the plasma membrane peaks.

In the present study, it was found that with purified microsomes, only one peak could be considered more characteristic of endoplasmic reticulum. It was surprising to find, however, that plasma membranes (mit) and purified microsomes could, in spite of quite different enzyme content, differ so little in their gel patterns. A possible explanation of this is that the peaks observed in dodecyl sulfate polyacrylamide gels are composite and that marker enzymes represent only a small fraction of the peptide peak. It should also be noted that dodecyl sulfate polyacrylamide gel electrophoresis, which allows separation of the peptides essentially according to their molecular weight [30], yielded gel patterns of plasma membranes (mit) and microsomes more similar to each other than did Triton X-100 polyacrylamide gel electrophoresis.

In this study, the effects of the mode of preparation of white adipose tissue

plasma membranes and microsomes upon some of their characteristics have been described. It is suggested that the choice of approach should depend upon whether the study's purpose is to get membranes with high enzyme activities or with a high degree of purity. It is further advised that the conditions of homogenization be carefully controlled due to the possible lability of microsomal enzymes.

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