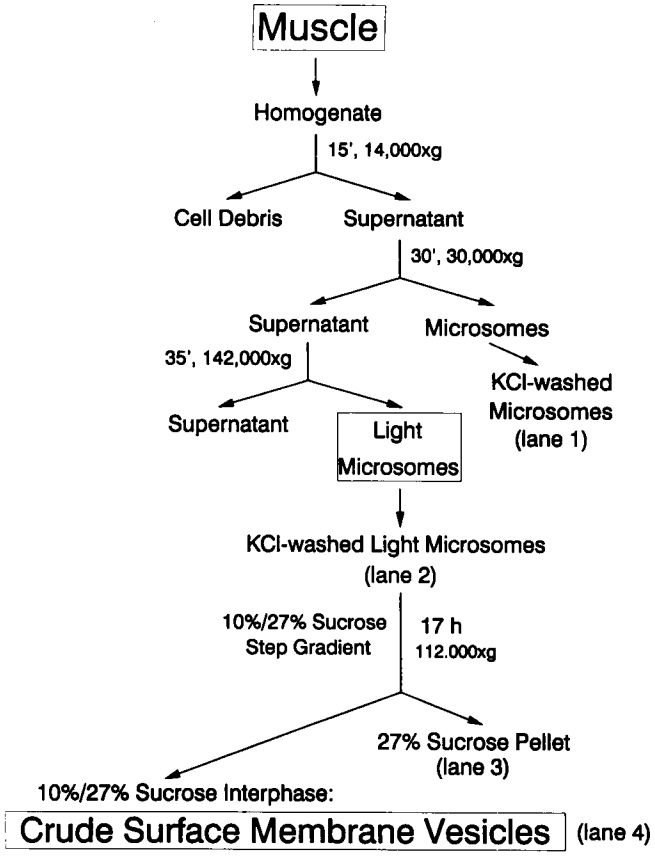

Figure 6. Flow chart of procedure to isolate crude surface membrane vesicles. KCl-washed light microsomes were prepared from rabbit skeletal muscle homogenate by differential centrifugation and subsequently crude surface membrane vesicles isolated by sucrose step gradient centrifugation. Next to the isolated fractions is the lane number in parentheses that will appear in the immunoblot analysis of Fig. 7.

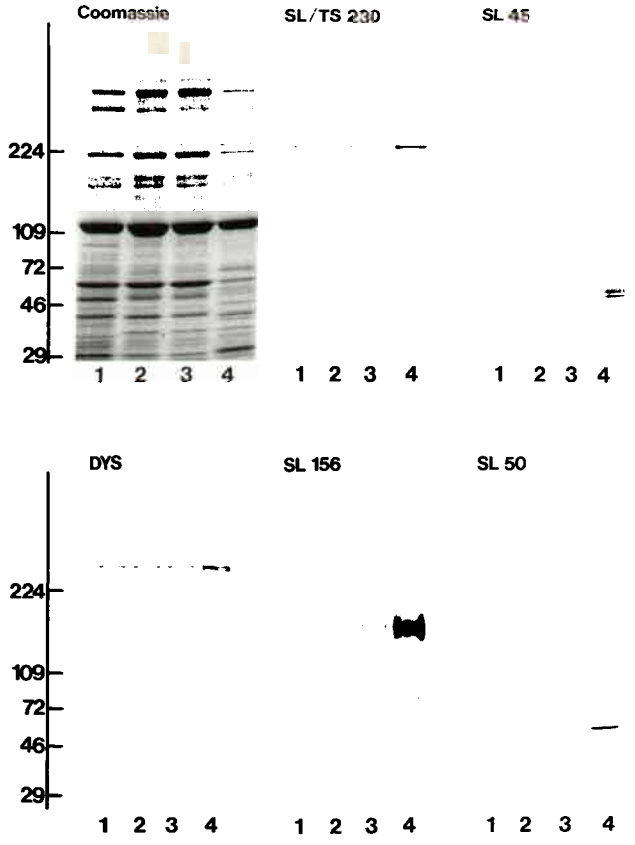

Figure 7. Immunoblot analysis of crude surface membrane preparation. Muscle membranes were prepared as described in Materials and Methods and separated by SDS-PAGE followed by immunoblotting with mAbs. Shown are a Coomassie blue-stained gel and identical immunoblots labeled with mAb $IXC1_2$ against SL/TS 230, mAb XVB9 against SL45, mAb XIXC2 against dystrophin, mAb $VIA4_1$ against SL156, and mAb $IVD3_1$ against SL50. Lanes *1–4* consist of KCl-washed microsomes, light microsomes, pellet from the sucrose-density step gradient and crude surface membranes from rabbit skeletal muscle (50 μg protein/lane). The molecular weight standards ($M_r \times 10^{-3}$) are indicated on the left.

(Fig. 10, *RyR*). mAb IIID5 to the α_1-subunit of the dihydropyridine receptor labeled strongly the transverse tubule fraction and also triads, but not the purified sarcolemma vesicles, indicating a good removal of t-system from sarcolemma in the WGA agglutination procedure. Furthermore, purified sarcolemma was stained with sarcolemma marker mAb XVB9, and mAb $IXC1_2$ labeled, in accordance with its dual immunofluorescence localization pattern, sarcolemma, transverse tubule, and triad vesicles (Fig. 10, *SL/TS230*). mAb McB2 to the Na^+/K^+-ATPase, which is generally considered a surface membrane marker, exhibited labeling of triads, transverse tubules and purified sarcolemma (Fig. 10, *Na/K-ATPase*). Immunofluorescence labeling of skeletal muscle cryosections did not show strong enough labeling to clarify the localization of this antigen.

The initial screening of the four subcellular fractions from rabbit skeletal muscle established the high degree of purity of the sarcolemma preparation used in this study. The preparation is essentially free of triads and transverse tubules and contains only small amounts of sarcoplasmic reticulum vesicles, much less than for example the transverse tubule preparation. The yield of crude and purified sarcolemma vesicles from 450 g of skeletal muscle was ~30 and 4 mg protein, respectively. This makes the WGA agglutination procedure described in this investigation a good method to isolate purified sarcolemma vesicles in a sufficiently high yield to characterize the components of the dystrophin–glycoprotein complex in plasma membrane vesicles.

Identical immunoblots, which were used for the initial screening of the above characterized subcellular fractions of rabbit skeletal muscle, were labeled with mAbs XIXC2, $VIA4_1$ and $IVD3_1$ against the dystrophin–glycoprotein complex (Fig. 10). Strong staining of the protein bands for dystrophin, the 156-kD glycoprotein and 50-kD glycoprotein were found in the purified sarcolemma fraction and some weak labeling in the transverse tubule fraction. These results agree with the immunofluorescence labeling of the cell periphery with the same mAbs (Fig. 2) and indicate that the components of the dystrophin–glycoprotein complex are