CHEMICAL CHARACTERIZATION OF ISOLATED EPIDERMAL DESMOSOMES

CHRISTINE J. SKERROW and A. GEDEON MATOLTSY

From the Department of Dermatology, Boston University School of Medicine, Boston, Massachusetts 02118

ABSTRACT

Desmosomes, isolated from cow nose epidermis by a method utilizing citrate buffer pH 2.6 and density gradient centrifugation, have been analyzed and found to contain approximately 76% protein, 17% carbohydrate, and 10% lipid. Nonpolar amino acids predominate in desmosomal protein, representing 456 residues per 1,000. The sialic acid content is 5 nM/mg of protein. The lipid fraction is composed of approximately 40% cholesterol and 60% phospholipids.

Desmosomes are completely solubilized by incubation with 2% sodium dodecyl sulphate and 1% β-mercaptoethanol. Gel electrophoresis of the denatured desmosomal proteins reveals 24 bands, with mobilities corresponding to a molecular weight range of 15,000–230,000 daltons. Seven of these are considered to be major bands, together constituting 81% of the desmosomal protein. Bands 1 and 2, of molecular weights 230,000 and 210,000 daltons, together comprise 28% by weight of the desmosome. It is suggested that these protein chains are located in the desmosomal plaque. Bands 3 and 4 are PAS-positive, constitute 23% of the desmosomal protein, and have apparent molecular weights of 140,000 and 120,000 daltons, respectively. At least part of this material must originate from the carbohydrate-containing layer which is demonstrated, by histochemistry, to be present in the desmosomal interspace. The possible nature and origin of the remaining major bands, of molecular weights 90,000, 75,000, and 60,000 daltons, are discussed.

Electron microscopy has shown that the desmosome consists of parallel unit membranes separated by an interspace which is bisected by a dense midplate and backed by a thick dense plaque. While plasma membranes have been extensively studied both *in situ* and in vitro by histochemical and biochemical methods, and a great deal of information has been obtained about their chemical composition, relatively little is known about the chemical nature of specializations of the plasma membrane such as the desmosome. Most of our present knowledge on desmosomes has been derived from histochemical studies. It has been noted in the light microscope that the nodes of Bizzozero, corresponding to epidermal desmosome sites, give a positive reaction for lipid, phospholipid, polysaccharide, and protein-bound sulfhydryl groups (14). Electron microscope histochemistry has shown the presence of acidic glycoproteins on the outer surface of the plasma membrane, including those regions participating in the desmosomal junction (9, 11, 16, 17). The presence of

sialic acid residues associated with calcium, in the desmosomal interspace, was demonstrated by the treatment of isolated plasma membranes containing a few desmosomes with EDTA and colloidal iron hydroxide (1).

A better understanding of the mechanisms whereby desmosomes form strong junctions between cells and become firmly bound to cytoplasmic filaments can be expected only by the acquisition of a more detailed knowledge of their chemical composition. Isolation of epidermal desmosomes by the use of citrate buffer pH 2.6 and density gradient centrifugation, as described in the accompanying paper (20), provides a new approach to study the chemical nature of these specialized regions of the cell periphery. Studies along these lines are presented in this paper.

MATERIALS AND METHODS

Desmosomes were isolated from cow nose epidermis, as has been described in the accompanying paper (20).

Gross Analysis

The following analyses were performed on desmosomes which were lyophilized and then dried to constant weight. Protein was determined by the method of Lowry et al. (10), using bovine serum albumin as a standard. Total neutral hexose was assayed by the Winzler orcinol-sulphuric acid method (23) using glucose standards. Sialic acid was estimated by the method of Warren (21), after solubilization of desmosomes by treatment with hydrochloric acid, pH 1.8, for 24 h at room temperature. Total lipids were extracted with chloroform-methanol in the ratio of 2:1. The extract was centrifuged at 30,000 g for 10 min and dried under a stream of nitrogen. The lipid was taken up in hexane, and 1-, 3-, 5-, and 10-μl samples spotted onto a plate for thin-layer chromatography. A standard containing cholesterol, oleic acid, triolein, and cholesterol oleate was also run. The plates were developed using hexane-ether-acetic acid (70:30:1). Values for total lipid, cholesterol, and total phospholipid contents were obtained by densitometry of the thin-layer chromatograms (4).

Amino Acid Analysis

Samples of lyophilized desmosomes from two different preparations were hydrolyzed under nitrogen in 6 N hydrochloric acid at 110°C for 24 and 48 h. The hydrochloric acid was removed *in vacuo* and the amino acid composition of the hydrolysates determined by automated ion exchange chromatography using the Jeolco amino acid analyzer (JEOL USA, Analytical Instrument Div., JEOL U. S. A., Cranford, N. J.).

SDS-Polyacrylamide Gel Electrophoresis

PREPARATION OF PROTEIN SOLUTIONS: Samples of both freshly prepared and lyophilized desmosomes were denatured by incubation at 100°C in 0.01 M sodium phosphate buffer pH 7.0, containing 1 mM ethylenediamine tetraacetic acid (EDTA), 1-2% sodium dodecyl sulphate (SDS), and 1% β-mercaptoethanol. The protein concentration was approximately 1 mg/ml. The denaturation time was 30 min for fresh desmosomes and 60 min for lyophilized desmosomes. After incubation of lyophilized desmosomes in 1% SDS, the solution was slightly cloudy and it cleared only on the further addition of SDS to a final concentration of 2%. Samples were dialyzed overnight against 0.01 M sodium phosphate buffer, pH 7.0, containing 1 mM EDTA, 0.1% SDS, and 0.1% β-mercaptoethanol.

Samples of rabbit myosin, phosphorylase a, bovine serum albumin, and ovalbumin were denatured for 30 min at 100°C, and run as molecular weight markers.

PREPARATION OF THE POLYACRYLAMIDE GELS: 5% and 10% fully cross-linked gels were prepared, essentially as described by Weber and Osborn (22). To improve resolution and the flatness of the bands, modifications (5) were introduced as follows: the denaturing buffer contained 1 mM EDTA. Electrophoresis buffer contained 2 mM EDTA, to give a final concentration of 1 mM in the gels. An unusually high ammonium persulphate-TEMED ratio was used, which extended polymerization time to approximately 30 min. Thus, 30 ml of gel solution contained 1.5 ml of ammonium persulphate solution at 30 mg/ml, and 7.5 μl of TEMED. Before polymerization, the gels were layered with a solution containing 0.1% SDS, 0.15% ammonium persulphate, and 0.05% TEMED in distilled water. After polymerization, the surface of each gel was rinsed and layered with electrophoresis buffer. The gels were then allowed to stand for at least 12 h before use.

The electrophoresis and staining with Coomassie blue was performed as described by Weber and Osborn (22), with 1 h staining time, at 37°C. Destaining was carried out in slotted tubes immersed in a large volume of destaining solution which was stirred vigorously and changed several times.

Staining for carbohydrate with the periodic acid-Schiff's reagent (PAS) was conducted as described by Fairbanks et al. (5). A duplicate gel was checked for nonspecific staining by omitting the periodic acid oxidation step.

SCANNING OF THE POLYACRYLAMIDE GELS: Destained gels were scanned with the Gilford 400S spectrophotometer with linear transport accessory (Gilford Instrument Laboratories, Inc., Oberlin Ohio), using wavelengths of 540 nm for Coomassie blue and 560 nm for PAS-stained gels. The contribution of each band to the total stain was assessed by cutting out and weighing the peaks. Gels prepared with several different

desmosome preparations and protein loadings were scanned and average values obtained.

RESULTS

Gross Analyses

The overall composition of isolated desmosomes was found to be approximately 76% protein, 17% neutral hexose, and 10% lipid. These are average values obtained from estimations on several different desmosome preparations. The lipid fraction was composed of approximately 40% cholesterol and 60% phospholipid, and only traces of neutral fats, triglycerides, and free fatty acids were present. The sialic acid content of isolated desmosomes was found to be 5.1 nM/mg of desmosomal protein.

Amino Acid Analysis

The amino acid composition of two desmosome preparations is shown in Table I. Little variation is seen between the preparations. It is noteworthy that desmosomes contain a very high proportion of nonpolar residues (456 residues per 1,000) of which 102 residues are leucine. The cysteine content of desmosomes is too low to be accurately measurable. Only those peaks corresponding to the amino acids listed in Table I were present on the chromatograms.

TABLE I

Amino Acid Composition of Desmosomes

	Preparation no. 4 (residues per 1,000)		Preparation no. 15 (residues per 1,000)	
	24 h	48 h	24 h	48 h
asp	101.6	102.8	101.7	102.2
thr	57.9	53.9	58.3	56.7
ser	70.6	66.1	74.3	66.8
glu	136.4	137.6	136.9	138.3
pro	45.4	47.6	45.8	48.4
gly	80.3	82.7	78.5	77.1
ala	73.8	68.9	73.1	73.2
val	63.9	65.5	64.2	66.4
½-cys	trace	trace	trace	trace
met	23.7	22.6	24.4	24.6
ile	55.8	57.8	56.1	57.7
leu	101.6	106.8	102.8	104.4
tyr	21.3	20.4	22.2	20.2
phe	34.9	34.5	35.9	36.8
lys	56.4	57.4	52.5	53.0
his	20.6	20.5	20.6	21.4
arg	55.9	54.3	52.5	52.2

SDS-Polyacrylamide Gel Electrophoresis

Electrophoresis of three different lyophilized desmosome preparations, with 5% gels, gave almost identical patterns in which 24 bands can be recognized after staining with Coomassie blue (Fig. 1). It was noted that the upper few millimeters of 5% gels contained a small amount of material which was completely excluded from 10% gels (Fig. 1). When fresh desmosome preparations are run, however, only a trace of this uppermost band is present. Lyophilized desmosome preparations show highly variable amounts of this material, up to a maximum of 10% of the total stain. A significant increase in the amount of the uppermost band, accompanied by spreading of band 4 and a decrease in the staining intensity of bands 1 and 2, is observed when EDTA or β-mercaptoethanol is omitted from the denaturing solution. On the basis of these observations, it is concluded that the uppermost band represents either partially denatured or aggregated desmosomal protein, possibly produced by the lyophilization procedure.

The contribution of each band to the total amount of stain present on the gel was assessed by quantitative densitometry (Fig. 2, Table II) which shows that seven major bands (1-7, Fig. 2) constitute 81% of the total stain. A band was considered to be major if it represented 2% or more of the total stain (Table II). Of the major bands (Figs. 1 and 2), bands 1, 3, 5, 6, and 7 appear to migrate as single species. Band 2 migrates as a doublet with a minor leading component. Band 4 is broad and diffuse, and the shape of the peak obtained by densitometry suggests the presence of at least four components.

After PAS staining, two bands can be seen on the gel. The slower moving band, which stains intensely, corresponds to band 3 on the Coomassie blue-stained gels. The faster moving band, which is composed of at least two poorly resolved components, can be identified with the slower components of band 4 visualized with Coomassie blue (Fig. 2). The relationship of PAS-positive bands to Coomassie blue-stained bands was determined by marking the PAS bands with Indian ink and subsequently staining the gel with Coomassie blue. On the densitometry trace of the gel stained with PAS, two additional small peaks, not visible to the naked eye, can be seen. They correspond to the Coomassie blue-stained material, previously discussed, in the uppermost part of the gel, and to the group of minor peaks which occur between bands 2
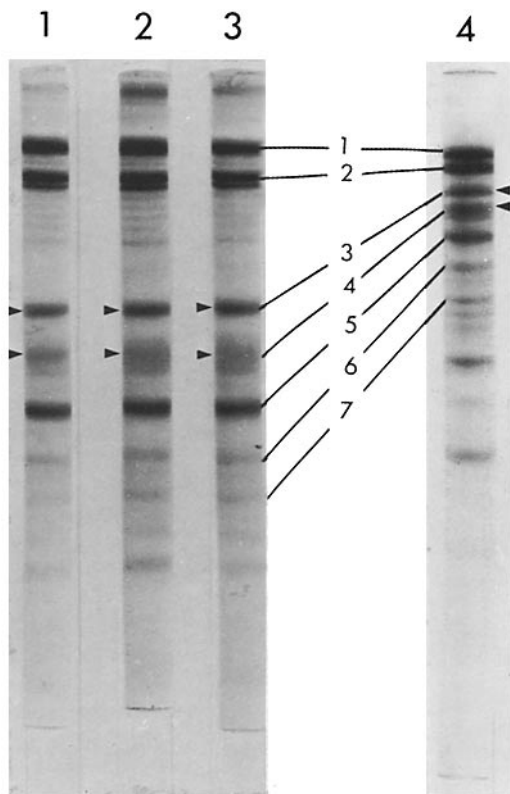
FIGURE 1 SDS-polyacrylamide gel electrophoresis of three preparations of desmosomes, using 5% acrylamide gels (gels 1–3). Gel 4 was prepared using a 10% acrylamide gel. All gels are fully cross-linked. Arrows point to PAS-positive bands.
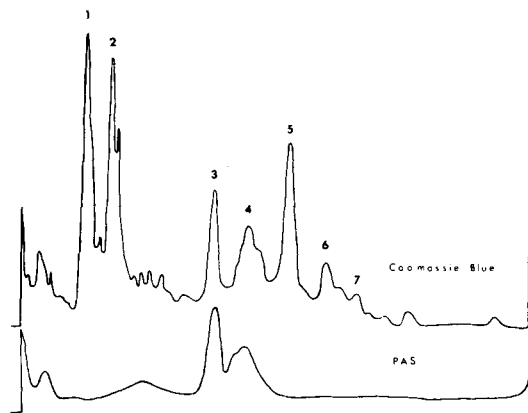

FIGURE 2 Densitometer traces of 5% SDS-polyacrylamide gels, stained with Coomassie blue and PAS reagent. The Coomassie blue-stained gel was scanned at 540 nm and the PAS-stained gel at 560 nm.

gel, only minor bands with molecular weights less than 15,000 daltons are observed.

and 3 (Figs. 1 and 2). None of these PAS-staining bands were observed when the periodic acid oxidation step was omitted.

From a calibration curve prepared with standard proteins (Fig. 3), the molecular weights of the major protein chains present in isolated desmosomes were found to range from 60,000 to 230,000 daltons (Table II). Values for bands 1 and 2 were obtained by a short extrapolation from the plot. As bands 3 and 4 contain glycoproteins, which behave anomalously on SDS gels, the apparent molecular weights given in Table II will be higher than the true values.

The lower limit of molecular weight detectable on 5% gels was found to be approximately 15,000 daltons. The possibility that desomsomes contain major protein components with molecular weights lower than this was examined by running desmosomes on a 10% gel (Fig. 1). However, on such a

DISCUSSION

The studies on isolated desmosomes presented in this paper provide general confirmation for previous histochemical data on in situ desmosomes, which demonstrated the presence of protein, lipid, and carbohydrate in this junctional complex (14). The histochemical information has been extended by quantitative determinations which show that protein is the major constituent of isolated desmosomes, amounting to approximately 76% of the dry weight. The presence of approximately 17% carbohydrate and 10% lipid in isolated desmosomes suggests that glycoproteins, lipoproteins, glycolipids, and mucopolysaccharides may also be components of the desmosomal junction.

The protein moiety of isolated desmosomes is characterized by an abundance of nonpolar amino acids, almost half of the amino acid residues being composed of leucine, isoleucine, valine, phenylalanine, glycine, alanine, and proline (Table I). Cysteine is present only in traces. The positive reaction for sulfhydryl groups noted in the nodes of Bizzozero (14) is therefore probably not related to desmosomes, but rather to the tonofibrils associated with the desmosomal plaque. Preparations of isolated desmosomes do not contain tonofibrils, as these are removed during the isolation procedure (20).

The large number of bands obtained by SDS-polyacrylamide gel electrophoresis of isolated des-

TABLE II

Size and Abundance of the Major Polypeptide Chains of Desmosomes

Band	Stain	
	Mol wt	%
1*	230,000	17.7 ± 1.8
2‡	210,000	20.4 ± 0.6
3§	140,000	8.9 ± 0.6
4‖	120,000	13.9 ± 1.7
5	90,000	13.2 ± 2.6
6	75,000	4.6 ± 1.4
7	60,000	2.2 ± 0.7
		80.9

* Obtained by a short extrapolation of the calibration curve.

‡ Band 2 is a doublet, with a minor, fast component of molecular weight 205,000 daltons. The value for percent of stain includes both components.

§ Bands 3 and 4 are PAS-positive, and apparent molecular weights are given.

‖ Band 4 is a mixture of at least four unresolved protein chains. The molecular weight given refers to the midpoint of the band.

mosomes indicates a level of complexity comparable to that shown by plasma membranes. Interpretation of the electrophoretic patterns in terms of specialized functions would be greatly facilitated by comparison with data on nonjunctional plasma membranes prepared from the same epidermis. Unfortunately, such preparations are not currently available. Accordingly, comparisons can be made at present only between the protein chains of isolated desmosomes and those reported in numerous studies of plasma membranes from other tissues (5, 6, 15). Common features include the presence of two bands of unusually high molecular weights, of the order of 200,000 daltons, PAS-positive bands of apparent molecular weight in the region of 130,000 daltons, and major bands of approximately 90,000, 75,000, and 60,000 daltons in molecular weight.

The two protein chains of high molecular weights (bands 1 and 2) together constitute 35% of the weight of the desmosome proteins, or 28% of the entire desmosome (Table II). Such chains are also present, in lower amounts, in the plasma membranes of liver and kidney cells, and are particularly prominent in erythrocyte ghosts, where they represent 25% of the ghost proteins or 12% of the entire ghost (5, 15). Proteins containing these chains have been isolated from the erythro-

cyte ghost and from liver plasma membranes, using low ionic strength reagents containing EDTA (3, 8). The most studied of these proteins, spectrin, which is derived from the erythrocyte ghost, is an "extrinsic" protein located on the cytoplasmic side of the membrane, where it forms a fibrous layer composed of 40-50-Å filaments (12). If bands 1 and 2, which together account for 28% of the entire desmosome, are similarly located, they would form a major part of the desmosomal plaque.

Spectrin and the analogous proteins in other plasma membranes are believed to form a fibrous network whose function is to support and strengthen the membrane structure (7, 8). The presence of unusually large amounts of such proteins in the desmosomal plaque would contribute greatly to the stability of the junction.

The proposition that the desmosomal plaque contains large amounts of a fibrous protein related to spectrin receives support from the observation that filaments of dimensions (40-50 Å) similar to those formed by spectrin are abundant in the plaques of both *in situ* (13) and isolated desmosomes (20). An alternative explanation, that these are actin filaments, is rendered improbable by the
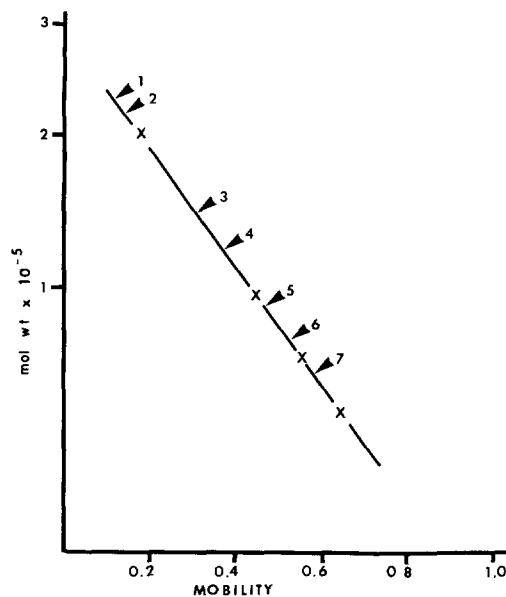

FIGURE 3 Calibration curve of molecular weight against mobility for 5% SDS-polyacrylamide gels. The standard proteins, in decreasing order of molecular weight, were: myosin, phosphorylase a, bovine serum albumin, and ovalbumin. Numbered arrows indicate major desmosome polypeptides.

small amount (0.6%) of protein of molecular weight 43,000 detected on gels of isolated desmosomes (Table II). In addition, the results of preliminary experiments conducted in this laboratory (19) indicate that after the extraction of isolated desmosomes with a low ionic strength reagent containing EDTA and β-mercaptoethanol, bands 1 and 2 are absent from the SDS-polyacrylamide gel.

The results of staining with the periodic acid-Schiff reagent show that bands 3 and 4 which together comprise 23% of the desmosomal proteins, both contain carbohydrate. Correlation of these data with electron microscope histochemistry suggests that a part of the desmosomal glycoproteins, represented by bands 3 and 4, occupies the desmosomal interspace. This is of particular interest as it is in this region that the specific interactions between cells which account for desmosomal adhesion presumably take place.

On the basis of histochemical evidence, Benedetti and Emmelot (1) suggested that cell-to-cell adhesion at the desmosome is due to the binding of calcium ions between sialic acid residues on adjacent cell surfaces. However, the results of this study show that while desmosomes contain considerable quantities of glycoproteins, their sialic acid content is very low (5 nM/mg) compared with that of most plasma membranes (50–100 nM/mg). It would therefore appear that a mechanism involving sialic acid residues may not be adequate to explain the enhanced adhesion at the desmosome, and alternative explanations, possibly involving other types of interaction between glycoproteins, should be sought.

Band 5, of molecular weight 90,000 daltons, constitutes 12% of the weight of the desmosomal protein, and, in terms of number of polypeptide chains, is the most abundant component. A band of this molecular weight is also a prominent component of many plasma membrane preparations (8). Protein chains with molecular weights of 75,000 and 60,000 daltons (bands 6 and 7) have also been observed in several plasma membrane preparations (5, 15). It cannot be concluded at present whether the desmosome and membrane bands of comparable molecular weights are identical or merely coincide in their mobilities. The possible identities and origins of the minor bands observed on SDS-polyacrylamide gels also cannot be assessed.

The relationship of the polypeptide chains identified by SDS-polyacrylamide gel electrophoresis to the native proteins of the desmosome must ultimately be clarified by isolation of intact proteins. Methods are known for the selective elution of proteins from isolated membranes (8) and also for the extraction of components from *in situ* desmosomes (2, 18). The use of such methods on isolated desmosomes, combined with electron microscope monitoring, should give more precise information on the location and function of the desmosomal components reported in the present study.

REFERENCES

1. BENEDETTI, E. L., and P. EMMELOT. 1968. Structure and function of plasma membranes isolated from liver. *In* The Membranes. A. J. Dalton and F. Hagenau, editors. Academic Press, Inc., New York. 33–120.
2. BORYSENKO, J. Z., and J. P. REVEL. 1973. Experimental manipulation of desmosome structure. *Am. J. Anat.* 137:403–422.
3. BRANDON, D. L. 1971. Purification of a 200,000 molecular weight membrane protein. *Fed. Proc.* 30:1065.
4. DOWNING, D. T. 1968. Photodensitometry in the thin-layer chromatographic analysis of neutral lipids. *J. Chromatogr.* 38:91–99.
5. FAIRBANKS, G., T. L. STECK, and D. F. H. WALLACH. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry.* 10:2606–2617.
6. GLOSSMANN, H., and D. M. NEVILLE. 1971. Glycoproteins of cell surfaces. *J. Biol. Chem.* 246:6339–6346.
7. GUIDOTTI, G. 1972. Membrane proteins. *Annu. Rev. Biochem.* 41:731–752.
8. JULIANO, R. L. 1973. The proteins of the erythrocyte membrane. *Biochim. Biophys. Acta.* 300:341–378.
9. KELLY, D. E. 1966. Fine structure of desmosomes,

hemidesmosomes, and an adepidermal globular layer in developing newt epidermis. *J. Cell Biol.* **28**:51–72.

10. LOWRY, O. H., N. J. ROSENBROUGH, A. L. FARR, and R. J. RANDAL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275.

11. LUFT, J. H. 1971. Ruthenium red and violet. II. Fine structural localization in animal tissues. *Anat. Rec.* **171**:369–415.

12. MARCHESI, V. T., and G. E. PALADE. 1967. The localization of Mg-Na-K-activated adenosine triphosphatase on red cell ghost membranes. *J. Cell Biol.* **35**:385–404.

13. McNUTT, N. S., and R. S. WEINSTEIN. 1973. Membrane ultrastructure at mammalian intercellular junctions. *Prog. Biophys. Mol. Biol.* **26**:45–101.

14. MONTAGNA, W. 1962. The Structure and Function of Skin. Academic Press, Inc., New York. 14–121.

15. NEVILLE, D. M., and H. GLOSSMANN. 1971. Plasma membrane protein subunit composition. *J. Biol. Chem.* **246**:6335–6338.

16. RAMBOURG, A. 1971. Morphological and histochemical aspects of glycoproteins at the surface of animal cells. *Int. Rev. Cytol.* **31**:57–114.

17. RAMBOURG, A., and C. P. LEBLOND. 1967. Electron microscope observations on the carbohydrate-rich cell coat present at the surface of cells in the rat. *J. Cell Biol.* **32**:27–33.

18. RASH, J. E., J. W. SHAY, and J. J. BIESELE. 1968. Urea extraction of Z bands, intercalated disks, and desmosomes. *J. Ultrastruct. Res.* **24**:181–189.

19. SKERROW, C. J. 1974. The Isolation and Characterization of Epidermal Desmosomes. Ph.D. Thesis. Boston University Graduate School, Boston, Mass.

20. SKERROW, C. J., and A. G. MATOLTSY. 1974. Isolation of epidermal desmosomes. *J. Cell Biol.* **63**:000.

21. WARREN, L. 1959. The thiobarbituric assay of sialic acids. *J. Biol. Chem.* **234**:1971–1975.

22. WEBER, K., and M. OSBORN. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**:4406–4412.

23. WINZLER, R. J. 1955. Determination of serum glycoproteins. *Methods Biochem. Anal.* **2**:279–311.