The Dispersal of Dermal Cells with Sodium Dodecyl Sulfate[1] (36399)

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Both rat skin and human dermis have been separated into their major morphologic components by sequential treatments—extraction with isotonic saline to remove the "ground substance"; digestion with trypsin to remove the cells; and autoclaving to disperse the collagen (1). However, trypsin results in contamination of the cellular fraction with telopeptides deleted from collagen (2). The reported ability of sodium dodecyl sulfate (SDS) to disperse tumor cells (3) suggested that this reagent might effectively disperse the cells of the skin.

Materials and Methods. Skin, shaved and freed of subcutaneous tissue, was obtained from male Sprague-Dawley rats (200 g). Human skin was collected from the grossly normal portions of legs amputated because of vascular insufficiency; subcutaneous connective tissue was removed by dissection and epidermis with a Stryker dermatome set at 0.01 in. Both tissues were minced, freeze-dried, and extracted with light petroleum and isotonic saline at 4°, as described elsewhere (1). In the experiments with fresh dermis, the freeze-drying and extraction with petroleum ether were omitted. Samples for histological examination were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin. Tissues were extracted with SDS at approximately 40° by placing an infrared lamp above the extractor (1). SDS was removed from extracts by dialysis against running tap water at 40° for 48 hr, followed by dialysis overnight against distilled water at 4°.

Ultraviolet absorption was measured in 1.00 cm quartz cells in a Beckman DU spectrophotometer. Extracts were analyzed for nitrogen (4), hydroxyproline (5), hexosamine (6), and hexuronic acid (7). Glycosaminoglycans (GG) were isolated by the method of Schiller *et al.* (8) modified by the omission of the extraction with alkali and by the substitution of *Streptomyces griseus* protease (Pronase Type B, Calbiochem) in 0.1 *M* Tris hydrochloride, pH 8.0, for trypsin in phosphate buffer. The GG were separated into nonsulfated (Fraction I) and monosulfated (Fraction II) fractions by elution from AG-1 (9). The monosulfated fractions were further separated into dermatan sulfate (fraction IIA) and other monosulfated GG (fraction IIB) portions by fractionation of their calcium salts with ethanol. Each GG fraction was characterized by analysis for hexosamine (6), hexuronic acid (10), and nitrogen (4). The hexosamine was identified by paper chromotography (11, 12) and the homogeneity of the GG was examined by electrophoresis (13, 14). These procedures will be described in greater detail elsewhere.

Results. Rat skin, previously dried, defatted and extracted with isotonic saline, was subjected to a variety of treatments thought likely to disperse the cells (Table I). Isotonic sodium chloride weakened the nuclear and cytoplasmic staining; some material absorbing at 280 nm was extracted. After treatment with distilled water, fibrocytes were apparent as degenerate nuclei; other cells were pale but intact. After repeated freezing and thawing, all cells except those of the stratum granulosum were shrunken and faded. Little ultraviolet-absorbing material was extracted by distilled water; freezing and thawing was equally ineffective. SDS in either 0.15 or 1 *M* NaCl removed all evidence of cellular

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TABLE I. Absorbance of Various Extracts of Rat Skin.[a]

Treatment	Extinction at 280 nm
0.15 *M* NaCl	10.8, 10.3, 10.9
Water	2.8, 2.5, 2.9
Water; freezing ×3	2.6, 2.6, 2.5
5 m*M* SDS/0.15 *M* NaCl	43.2, 38.2, 36.3
5m*M* SDS/1 *M* NaCl	43.2, 43.2, 41.2

[a] Triplicate residues representing 1 g of rat skin, were extracted three times for 2 hr with 5 ml of the various reagents. After centrifugation, the supernates were filtered through glass wool, pooled and diluted to 20 ml. The absorbance was multiplied by [vol of extract (ml)]/[wt of sample (g)].

material except for an occasional nuclear remnant in the hair follicles and stratum granulosum. The ultraviolet absorption of the SDS extracts was four times that of extracts prepared with isotonic saline and 15 times that of those prepared with distilled water.

Extractants containing SDS were examined in greater detail using fresh human dermis as test material (Table II). Isotonic saline was used as a control; molar sodium chloride, to enhance the solubility of the nucleoproteins. As judged by the nitrogen analyses and ultraviolet absorption of the undialyzed extract, 1 *M* NaCl was a more effective extractant than 0.15 *M* NaCl. However, the dry weight and nitrogen analyses after dialysis differed little between the two treatments. SDS increased sharply all measures of extracted material; 1 *M* NaCl did not enhance its effect. If judged by the dry weight and total nitrogen of the dialyzed extract, salt somewhat depressed the ability of the detergent to disperse tissue components. Although 1 *M* NaCl dispersed most of the cells except for those of the remaining epidermal fragments, the action of the detergent was more consistent and complete. After exposure to SDS, only a few cellular remnants could be detected among the epidermal fragments (Fig. 1). If the nondialyzable material is assumed to comprise protein containing 16% nitrogen, a value of 300 μmoles would correspond to 26 mg accounting for most, but not all, of the weight. Thus, both the histological and the analytical data revealed that SDS in distilled water effectively dispersed the cells of the dermis.

The study of SDS was undertaken to examine the extent to which collagen telopeptides influenced the composition of the tryp-

TABLE II. A Comparison of Various Extractants of Human Dermis.[a]

Extractant	Dry wt (mg/g of skin)	Extinction at 280 nm	Nitrogen (μmole/g) Before dialysis	Nitrogen (μmole/g) After dialysis
0.15 *M* NaCl	18.2	10.4	103	76
	13.9	11.5	111	68
	8.7	12.0	118	49
1 *M* NaCl	14.9	15.4	170	72
	7.8	15.8	171	84
	20.2	17.2	188	88
5 m*M* SDS	41.8	24.0	356	297
	46.7	—	—	275
	23.3	25.6	393	303
5 m*M* SDS/1*M* NaCl	30.5	25.8	399	208
	32.2	22.1	361	208
	28.0	23.0	374	216

[a] Triplicate residues from 1.0 g of fresh human dermis were extracted four times for 90 min with 5 ml of the extractant. After centrifugation, the supernates were filtered and pooled. The volume was measured, a portion was used for nitrogen analysis and for the measurement of ultraviolet absorption. The remainder was dialyzed to remove SDS, freeze-dried and weighed. The dry residue was dissolved in 0.01 *N* NaOH and analyzed for nitrogen.

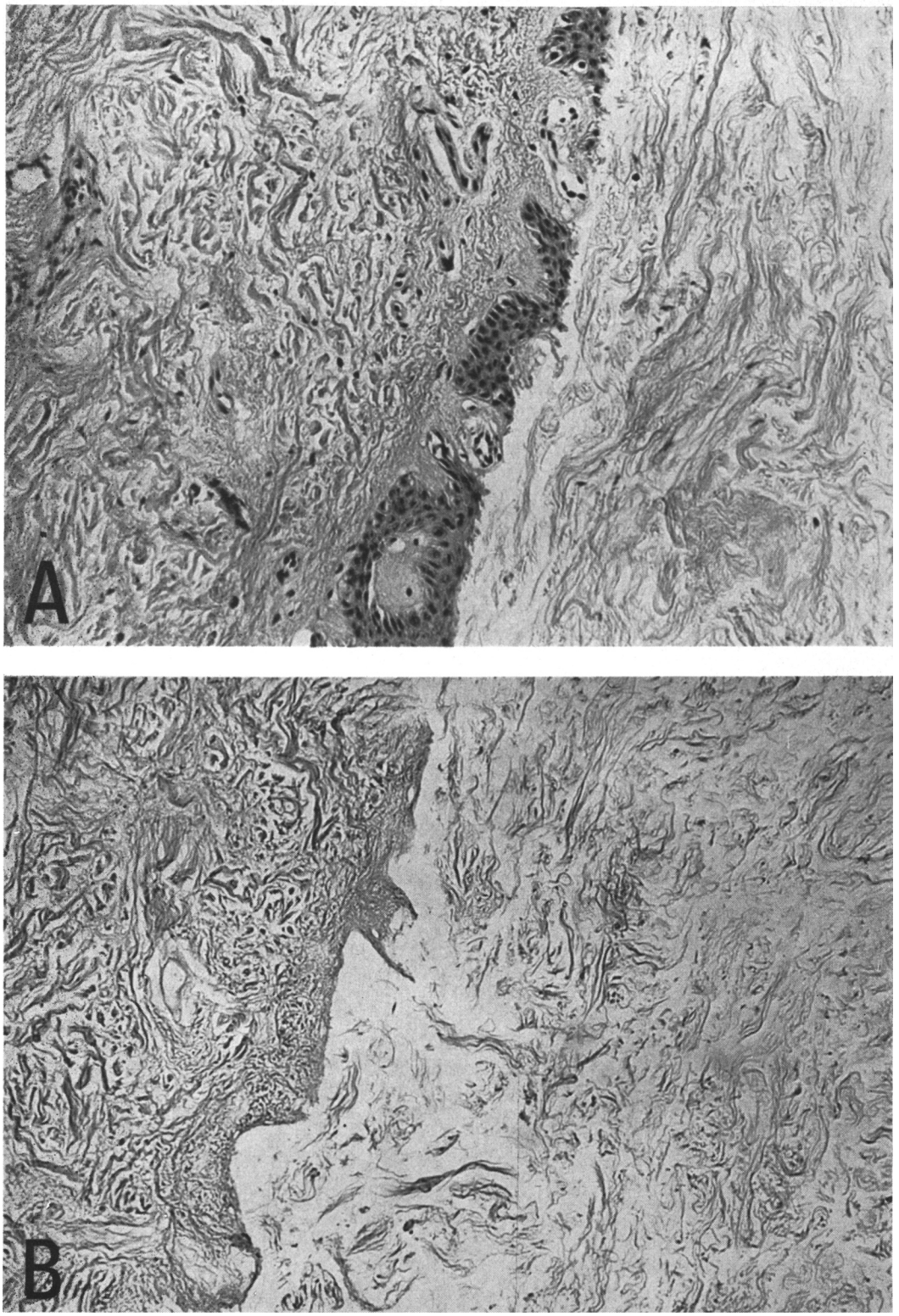

FIG. 1. Photomicrographs of minced human dermis, H & E, × 300. (A) After freeze-drying and extraction, in succession, with light petroleum and isotonic saline. (B) After four further 90 min treatments with 5 m*M* SDS.

TABLE III. SDS Fractions Compared with Trypsin Fractions.[a]

Component	Mean ± SD (μmole/g of fresh wt)	
	Found in SDS	Calculated for trypsin
Nitrogen	129 ± 34	221 ± 38
Hydroxyproline	6.09 ± 2.28	6.34 ± 2.43
Hexosamine	1.68 ± 0.26	1.39 ± 0.30
Hexuronic acid	0.74 ± 0.08	1.14 ± 0.36

[a] The data for the SDS fractions were obtained from five human legs; those for trypsin, from the skin of the thorax of 13 individuals, to be described in detail elsewhere.

sin fraction. Analyses of SDS extracts of five samples of human dermis were compared with results previously obtained by trypsin extraction of similar tissue. With the exception of nitrogen, analyses of the two types of extract were strikingly similar (Table III). The trypsin fractions averaged 100 μmoles of nitrogen/g of fresh wt more than the SDS fractions. Since the 10 mg of trypsin used to prepare the trypsin extract contained 114 μmoles of nitrogen, assuming 16% nitrogen, the enzyme accounts for the difference in composition. Thus, the removal of cells from human dermis by either trypsin or SDS yielded fractions of closely similar composition except for the protein contributed by the enzyme.

The glycosaminoglycans of the SDS fraction were isolated, fractionated and characterized (Table IV). Fraction I contained a 25% apparent molar excess of hexuronic acid over hexosamine, and showed glucosamine as the sole hexosamine on paper chromatography. Hyaluronate was the principal component on zone electrophoresis. Each of these properties is characteristic of hyaluronate. The slight contamination with dermatan sulfate apparent on electrophoresis was insufficient to show galactosamine on paper chromatography. Fraction IIA showed galactosamine only on chromatography, an apparent hexuronic acid: hexosamine ratio of 0.6, and dermatan sulfate only on electrophoresis, corresponding in each of these respects to the usual properties of dermal dermatan sulfate. Fraction IIB contained both glucosamine and galactosamine, apparently equimolar amounts of hexuronic acid and hexosamine, and three distinct bands on electrophoresis. This fraction is obviously a complex mixture.

The yield of dermatan sulfate exceeded considerably that of either hyaluronate or the mixed fraction. The values corresponded closely to those found for the trypsin fraction in other subjects—0.04, 0.27 and 0.10 μmoles of hexosamine/g of fresh wt for Fractions I, IIA and B, respectively. The glycosaminoglycans of the detergent fraction thus correspond both in chemical constitution and yield to those of the trypsin fraction.

Discussion. The concentration of SDS used for extraction was the minimal level reportedly required to disperse nuclei, the most resistant cellular component visible in the

TABLE IV. Glycosaminoglycans of the SDS Fractions.[a]

Fraction	Yield	HexCOOH/ $HexNH_2$	$HexNH_2$		Electrophoresis		
			Glc	Gal	H	ChS	DS
I	0.055 ±0.028(5)	1.29 ±0.08(5)	5/5	0/5	5/5	0/5	3±/5
IIA	0.28 ±0.09(5)	0.57 ±0.02(5)	0/5	5/5	0/5	0/5	5/5
IIB	0.057 ±0.023(5)	1.00 ±0.07(5)	5/5	5/5	5/5	5/5	5/5

[a] The mean ± standard deviation (no. of specimens) is given for the yield (μmoles of hexosamine/g of fresh wt) and for μmole of hexuronic acid (HexCOOH) per μmole of hexosamine ($HexNH_2$). The (no. of positive results)/(no. of samples studied) is given for hexosamine chromatography (Glc = glucosamine; Gal = galactosamine) and paper electrophoresis (H = hyaluronate; ChS = chondroitin sulfate; DS = dermatan sulfate; ± = trace).

light microscope (3). The volume, duration and number of treatments were based on past experience with connective tissues (1), not on systematic experimentation. Being both effective and convenient, the procedure was deemed adequate.

Cellular constituents recognizable with a light microscope were removed by SDS. Convincing data supporting the complete removal of a morphologically defined entity from a tissue can only be achieved with great difficulty. Ultrastructural studies might have revealed mitochondria, endoplasmic reticulum, nucleoli and other cellular organelles not readily visible in the light microscope. Soluble constituents of the cytosol may have remained bound to the collagenous and elastic fibers of the residue. Although the presence of cellular constituents in either the saline extract or the residual insoluble fibers has not been rigorously excluded, the close agreement between the compositions of the two types of extract supports the claim that each represents the cells of the tissue. The contribution of the collagen telopeptides to the trypsin extract appears to have been negligible. Since the binding between SDS and proteins, including collagen and elastin, appears to be reversible (15, 16), the residue should be usable for the study of the scleroproteins of the dermis.

Summary. SDS (5 m*M*) removes histologically detectable cells from skin previously freed of ground substance with isotonic saline. Such extracts correspond in composition to those produced by trypsin.

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