SEPARATION OF EPIDERMIS FOR THE STUDY OF EPIDERMAL SULFHYDRYL*

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Quantitative determinations of the sulfhydryl and disulfide concentration of stratum corneum have shown relatively consistent values in certain skin diseases. Research in this interesting field has been more or less limited to diseases in which there is considerable scaling or marked thickening of the stratum corneum. Since changes in the stratum corneum are dependent upon the status of the underlying epidermal cells, studies of the complete epidermis might be quite informative. Iakovlev (1) has shown that sulfhydryl and disulfide groups can be determined in very small amounts of tissue, as little as 0.8 mg., by a microtechnic employing amperometric titration. Therefore, it appeared that this method could be adapted for the study of punch biopsy specimens if the epidermis could be satisfactorily separated from dermis.

In previous studies we determined the sulfhydryl and disulfide concentration of scales from patients with psoriasis and other scaling dermatoses by using the amperometric titration technic, and we found this method to be excellent (2, 3). Before the microtechnic could be employed, it became apparent that the various methods of detaching epidermis from dermis needed to be examined. The objective was to find the best procedure to use when —SH and S—S were to be determined on small specimens such as are obtained by punch biopsy.

Several means for freeing the epidermis from the corium have been described (4–10). These methods have included the utilization of trypsin digestion, heat, ionic change, suction, and mechanical separation, and each has advantages and disadvantages. Heat is simple, yet tedious. Although the heat process has been used successfully, there is a marked decrease in oxygen consumption by the epidermis (11). Chemical reagents, such as acetic acid, sodium carbonate and ammonium hydroxide are effective but disturb the electrolyte equilibrium of the cell. Digestion by trypsin gives complete separation, but it destroys some important enzymes. Mechanical division necessitates a relatively large piece of tissue but has the advantage that chemical changes do not occur. The separation method must be appropriate for the experimental project, and no single method appears to be superior for all purposes.

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METHODS FOR SEPARATION OF EPIDERMIS AND DERMIS

A. *Enzyme Digestion* (*4–9*)

The enzyme technic of disengaging epidermis from dermis has been employed by many investigators (4–9). The exact mechanism of separation has not been clearly established, although the epidermal sheet can be freed from the dermis by crude trypsin or pancreatin. The detaching action of pancreatic extract has been related by some to the elastase contained in crude pancreatin (6–8). In 1958, Fan (9) showed that epidermis could be separated with equal or smaller amounts of crystalline trypsin or purified trypsin than with pancreatin. Of the three agents tested, purified trypsin freed epidermis the most efficiently. There is very little elastase in trypsin; therefore, the action of enzyme in loosening epidermis is not dependent upon the action of elastase.

Fan's method (*9*)

Pancreatin (0.5% U. S. P.) and purified trypsin (0.5% Difco 1:250) are dissolved in an isotonic solution consisting of NaCl 0.042 gm., KCl 0.42 gm., $CaCl_2$ 4.20 gm., $NaHCO_3$ 0.15 gm., and glucose 0.1 gm. per 100 ml. of distilled water. The skin is incubated with the solution at 40° C, and detachment begins in 30 minutes.

B. *Heat* (*10, 11*)

In 1942, Baumberger *et al.* (11) placed skin on a slide warming table at 50° C for 2 minutes; after which, the epidermis could be peeled off with forceps. Incomplete separation was possible at 48.2°C, but complete division occurred with ease and completeness at 49.2° C. Separation appears to be due to a general softening of

collagen. However, if the temperature is higher than 51° C, difficulty is encountered in detachment.

C. *Ionic Change (11–17)*

It has been known for a long time that, after treating the skin with acetic acid, epidermis can be easily freed from corium. Alkaline solutions, such as lime water or an ammonium solution, are used in the leather industry to remove hair and epidermis from animal hides. In 1947, Felsher (12) reported in detail on the adherence of epidermis to the corium. Separation of the epidermis from the corium is effected by acids and bases at such a pH as will cause swelling of collagen.

Baumberger's method (11)

Human skin is soaked in normal NH_4OH for 20 minutes at room temperature, after which the epidermis can be loosened easily and completely from the dermis.

Griesemer's method (13)

The skin is dissected from underlying tissue and placed in ice cold 0.015 M potassium chloride. With the epidermis up, the skin is spread out on a board and held by thumbtacks. The epidermis is cleaned by rubbing it with cotton dipped in cold KCl. Shavings of epidermis are scraped off with a razor, the handle of which is unscrewed about one-quarter turn. These shavings are 90 to 150 microns thick and contain epidermis, a little dermis, and some sebaceous glands and ducts, but no hair follicles or large blood vessels.

Hollo's method (14–17)

Epidermis is disengaged from connective tissue by means of a normal solution of ammonium hydroxide or potassium hydroxide (14–17). Separation is far more rapid with potassium hydroxide solution.

D. *Suction Method (18)*

In 1950, Blank freed epidermis from dermis by producing bullae. A piece of human skin is tightly wired across the top of a heavy-walled thistle tube (3 cm.) so that the dermal side is outside. The tube is inverted and the dermal side of the skin is allowed to dip into water, but the edge of the piece of skin is kept above the water level. The tube is then attached to a vacuum pump. Water does not visibly pass through the skin; however, after a period of time, bullae begin to form on the skin. If pressure within the thistle tube is held at approximately 150 mm. of mercury, the required time for the bullae to form is from one to two hours. When the pressure is reduced to 10 mm. of mercury, bullae sometimes form in 15 to 20 minutes. Separation occurs at the dermal-epidermal junction.

E. *Mechanical Method (19, 20)*

Van Scott's method

In 1952, Van Scott recommended a stretching method for separation. This is rapid, simple, and has few objections, except that a sizeable piece of tissue is required. It can be performed on either fresh or previously frozen skin. A width of skin (0.5 to 1.5 cm.) is cut to any convenient length and the subcutaneous fat is removed. The skin is then manually stretched to its limit over a slightly convex wooden surface, and is anchored in place by means of thumbtacks. A razor blade or scalpel is used to scrape the epidermis free of the corium at one end, then the freed epidermis is grasped with a forceps and the whole epidermis is gently detached in a continuous sheet. The sharp blade can be used to free the epidermis in those places where it may tend to adhere. An alternative procedure is to remove the epidermis along its entire length simply by scraping.

Tabachnick's method (20)

The excised skin is stretched and tacked down on a piece of stiff cardboard. The skin is wiped with gauze soaked in ether, then hair is removed with an electric shaver. Epidermis is peeled off from the underlying dermis by using the scalpel and scraping. The major portion of removed material is made up of small sheets of intact epidermis.

COMPARATIVE STUDIES

A. *Materials and Methods*

Human skin from the mid-abdominal region was obtained from autopsy material. After removing the subcutaneous fat, epidermis was divided from the corium by means of Fan's trypsin digestion, Baumberger's heat method, Baumberger's ammonium method, and Van Scott's mechanical method, respectively. The detached specimens of epidermis were minced with scissors and defatted by washing with ether. This material was stored

in a desiccator and later finely pulverized in a porcelain mortar. The sulfhydryl concentration of each specimen was later determined by amperometric titration after incubating with redistilled water for one hour at 50° C. Sulfhydryl values are expressed as times 10^{-2} mM in 100 gm. dry tissue.

B. *Results*

Determinations on epidermis from fresh autopsy material found that the sulfhydryl values obtained following NH_4OH separation were quite similar to those found after mechanical separation. Enzyme and heat separation results were significantly different. Results are shown in Table 1.

TABLE 1

Effect of different separation methods on the sulfhydryl concentration of separated epidermis

Method.....	Julin's Method	Baumberger's Method	Baumberger's Method	Van Scott's Method
Principle.....	Enzyme	Heat	Ionic change	Mechanical
Procedure.....	Soak in enzyme solution for 30 min. at 40°C	Place on a hot plate (50°C) 2 minutes	Soak in 1N NH_4OH for 20 min.	Stretch and gently tear off
Determination of SH ($\times 10^{-2}$ mM per 100 gm)	83.0	82.0	81.0	81.0
	87.0	90.0	82.0	84.5
	81.0	87.0	70.0	72.0
	—	88.0	57.0	—
	—	105.0	97.0	—
	135.0	124.0	—	—
	55.0	58.0	49.0	50.0
	—	—	46.0	47.0

PRESERVATION OF TISSUE

Since biopsy specimens were to be taken in dermatology clinics, it became apparent that the most satisfactory method for preserving tissue needed to be known. First, the tissue had to arrive in the laboratory in a state which would allow separation. Second, the sulfhydryl concentration should be unchanged.

A. *Effect of Fixation Solution on Epidermal-Dermal Separation*

The skin was placed in acetone, alcohol, ether, formalin, or saline solution. All specimens were kept in solution for identical periods of time, then frozen for storage, and later, separation was performed by each method. The results are shown in Table 2. Only fresh skin and skin kept in saline solution can be separated satisfactorily by all methods.

B. *Effect of Different Fixation Solutions on Sulfhydryl Determinations*

The effect of different fixation solutions on sulfhydryl concentration of separated epidermis and on psoriasis scales was studied. Scales were placed in acetone, alcohol, ether, formalin, or saline solution for 20 hours at room temperature. At the end of this time, they were removed from the solution and the sulfhydryl concentration was determined.

From the results given in Table 3, the apparent sulfhydryl concentration of scale was found to

TABLE 2

Fixation solution and the separation process

	Heat	NH_4OH			Enzyme		
		20 min.	60 min.	120 min.	30 min.	60 min.	120 min.
Acetone	None*	None	Incomplete	Complete (difficult)	None	Complete (difficult)	Complete
Alcohol	None	None	Incomplete	Complete (difficult)	None	Complete (difficult)	Complete
Ether	Partial	None	Complete (difficult)	Complete	Complete	—	—
Formalin	None	None	None	None	None	None	None
Saline	Complete	Complete	—	—	Complete	—	—
Frozen	Complete	Complete	—	—	Complete	—	—

* none = no separation

TABLE 3

Effect of different fixation solutions on the sulfhydryl concentration of psoratic scale

Fixation Solution	SH Concentration*			Average
Acetone	22.3	20.6	18.3	20.4
Alcohol	22.0	21.7	18.1	20.6
Ether	22.0	20.6	19.1	20.6
Formalin	15.4	15.6	15.0	15.2
Saline solution	40.2	40.1	42.3	40.8
Control (Fresh Scale).	56.5	55.5	47.0	53.0

* SH expressed as $\times 10^{-2}$ mM per 100 gm.

TABLE 4

Effect of the actual separation process on the sulfhydryl concentration of epidermis

	After Autopsy	After 20 Hours			
Separation Method.....	Mechanical	Ammonium Method			
Preservation of Tissue....	Fresh	Fresh	Frozen	Saline	Refrig.
Determination of SH*	50.0	49.0	—	42.0	—
	47.2	45.5	47.5	40.0	45.8
	—	—	98.0	94.0	—

* SH expressed as $\times 10^{-2}$ mM per 100 gm.

vary with the nature of the solution. When compared to fresh scales, it can be seen that the sulfhydryl concentration was lowered by each solution. Saline was the closest to normal, and formalin the most changed.

Both frozen skin and skin kept in saline solution for 20 hours were separated by means of the ammonium method and the results are shown in Table 4. Frozen skin yielded results almost identical to those of fresh, mechanically separated skin, but saline caused some loss of sulfhydryl.

DISCUSSION

When selecting the method to be employed for separating epidermis, more things have to be considered than morphological completeness of separation. This is particularly true when biochemical determinations are to be performed. Mechanical and suction methods have a distinct advantage over other methods in that the skin is not subjected to heat, chemicals, or enzymes. Determinations on epidermis separated by the mechanical method should probably be regarded as the true values and allowed to serve as a basis for evaluating and comparing other methods. This method requires a large amount of skin and is not adaptable for clinical studies on routine patients. In this investigation, mechanically separated specimens were used to establish true values. Comparative studies then found that sulfhydryl values obtained after ammonium separation were quite similar to those obtained following mechanical separation, while heat and enzymes appeared to alter the results significantly and were therefore not satisfactory.

When biopsy specimens are taken in a clinic, tissue must be preserved for a period before it arrives in the laboratory. These studies show that if skin is placed in acetone, alcohol, ether, or formalin the epidermis cannot be freed as satisfactorily. Also, when the skin specimen is obtained from a cadaver that has been injected with formalin, separation is difficult.

Table 3 shows the effect of various solutions on the sulfhydryl concentration of scale. Psoriasis scale was chosen for this experiment, and it was found that the sulfhydryl concentration definitely varied with the nature of the solution. Saline caused a relatively small loss of sulfhydryl, and it is possible that skin specimens could be temporarily placed in saline solution without significant change.

In 1957, Kolthoff (21) demonstrated that the sulfhydryl of native albumin is not oxidized in 24 hours by oxygen in a buffer of pH 7 or 9, but it is oxidized by denaturation. Therefore, it is important that neither preservation nor separation result in denaturation of protein.

SUMMARY

1. The means for separating epidermis from the dermis were reviewed.

2. Selected methods were studied to determine the best procedure for detaching small pieces of skin without changing sulfhydryl values.

3. The sulfhydryl concentration in separated epidermis was determined by amperometric titration.

4. Baumberger's ammonium separation technic appeared to be the procedure of choice.

5. Freezing was found to be the best method for preserving tissue after biopsy removal.

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