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Biochemical Composition of Suction Blister Fluid Determined by High Resolution Multicomponent Analysis (Capillary Gas Chromatography—Mass Spectrometry and Two-Dimensional Electrophoresis)

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The biochemical composition of blister fluid was compared with serum and with blister fluid from erythematous lesions induced by ultraviolet irradiation.

By using glass capillary gas chromatography—mass spectrometry over 100 metabolites were determined and with the aid of two-dimensional high resolution electrophoresis (the ISO-DALT system) several hundred protein spots were seen.

The results show that the suction blister fluid qualitatively have a serum-like pattern but that the concentration of each compound was smaller than in serum. Also in suction blisters raised on erythematous reactions induced by ultraviolet light the same pattern was seen. The content of sodium, potassium and chloride was the same in suction blisters raised on erythematous and normal skin as that of serum.

The formation of subepidermal blisters was first recorded during cupping 100 yr ago [1]. However, the suction blister method was first described in detail by Kiistala and Mustakallio in 1967 [2]. They found electronmicroscopically that all suction blisters were cleanly subepidermal and that the level of separation was between the cell membranes of basal cells and the epidermal basement membrane. Today the suction blister method is widely used for dermatological research work (e.g., see 3).

The suction blister technique is a very useful *in vivo* model for evaluating the influence of external physical and chemical stimuli on the skin and especially for studying pathophysiology of inflammation. Cellular components released following external stimuli which may labilize or disrupt membranes or otherwise damage cells, may be collected in the suction blisters.

Some biochemical features of the suction blister fluid have already been documented [3–5]. The purpose of the present study was to determine the biochemical composition of blister fluid as compared to serum using advanced multicomponent analytical methods such as capillary gas chromatography—mass spectrometry (GC-MS) and two-dimensional electrophoresis. Also, the content of sodium, potassium and chloride was determined in order to reveal if intracellular electrolytes were released during the suction blister formation. The results show that blister fluid has a serum-like pattern of both low molecular weight metabolites and proteins.

MATERIALS AND METHODS

Subjects

Electrolytes were determined in suction blister fluid of 11 patients. The metabolite and protein pattern were examined in suction blister fluid before and after irradiation and in serum of the same 2 subjects. All the patients had only minor localized skin disorders on the extremities or face and with macroscopically normal abdominal skin. None of them had any systemic disease or were receiving systemic drugs.

Ultraviolet Injury

Abdominal skin was irradiated with an Osram High Pressure Xenon Arc Lamp (XBO = 150 W) equipped with the Schott filters $KG_1 + 310$ giving middle wave ultraviolet light (UVB). The transmission characteristics as measured spectrophotometrically showed half bandwidth 305–315 nm and tenth bandwidth 301–317 nm. The Xenon lamp was placed at a distance of 20 cm from the skin and the test area was about 1 cm^2. The individual minimal erythemal dose (MED) was determined in two subjects. Fresh test sites were subsequently irradiated with 3 times the MED and 24 hr later suction blisters were raised on these inflammatory sites.

Suction Blister Formation

One suction cup was placed on normal abdominal skin and one suction cup was placed with the bore (12 mm in diameter) in the adapter plate of the cups exactly over the inflammatory test site. The cups were made of transparent plastic allowing continuous observation of the blister formation. The cups were coupled in series. A suction pressure of 250 mmHg was provided by an electric vacuum pump (Air-Shields Dia-Pump (.U.K.) LTD. Towerfield, Estate, Shoeburyness, Essex). The negative pressure was measured with a mercury manometer (Air-Shields, INC. Hartboro, P.A., USA). The blisters were raised at room temperature (20–22°C) with the patient lying supine throughout.

Collection of Fluid

The suction blisters were punctured with a needle and the fluid collected in a 1 ml syringe. A venous blood sample was drawn from the subjects during the suction blister formation. Both blister fluid and blood samples were centrifuged at 3000 rpm for 10 min. The sodium, potassium and chloride content was determined within 2 hr. Suction blister fluid and serum samples were stored at −20°C in a deep freezer until further analysis within 1 week.

Electrolytes in Suction Blister Fluid

The determination of Na^+ and K^+ was done with a Flame Photometer (IL 343) and of Cl^- with Coulombmetric titration (Corning EEL 920). Suction blister fluid samples were collected from unirradiated skin and from skin irradiated with 3 times the MED of UVB 8 and 24 h previously.

Determination of the Metabolite Pattern by Glass Capillary Gas Chromatography—Mass Spectrometry

The serum or blister fluid (330 μl) was evaporated to dryness under nitrogen. Methanol (2 ml containing 2 M HCl) was added to the residue and the mixture was kept at 80°C over night to complete the methanolysis. After evaporation of the methanol/HCl a silylating reagent (50 μl of BSTFA) was added and the mixture was kept at 80°C for 20 min. This method of derivative formation leads to methylation of the organic acids, free fatty acids and yields methylglycosides with many carbohy-

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Abbreviations:
SDS: sodium dodecyl sulfate
TMS: trimethylsilyl

Na^+, K^+ and Cl^- content in suction blisters and serum

Suction blister fluid from	Na^+		K^+		Cl^-	
	Mean value	Range	Mean value	Range	Mean value	Range
Unirradiated skin (9 investigated subjects)	141 mmol/l	(135–151)	4.4 mmol/l	(3.8–4.9)	110 mmol/l	(99–117)
8 hr after irradiation (7 investigated subjects)	146 mmol/l	(136–165)	4.6 mmol/l	(3.9–6.1)	118 mmol/l	(107–128)
24 hr after irradiation (7 investigated subjects)	140 mmol/l	(136–144)	4.5 mmol/l	(4.0–5.0)	108 mmol/l	(105–111)
Serum (6 investigated subjects)	141 mmol/l	(135–150)	4.3 mmol/l	(3.8–4.7)	108 mmol/l	(102–112)

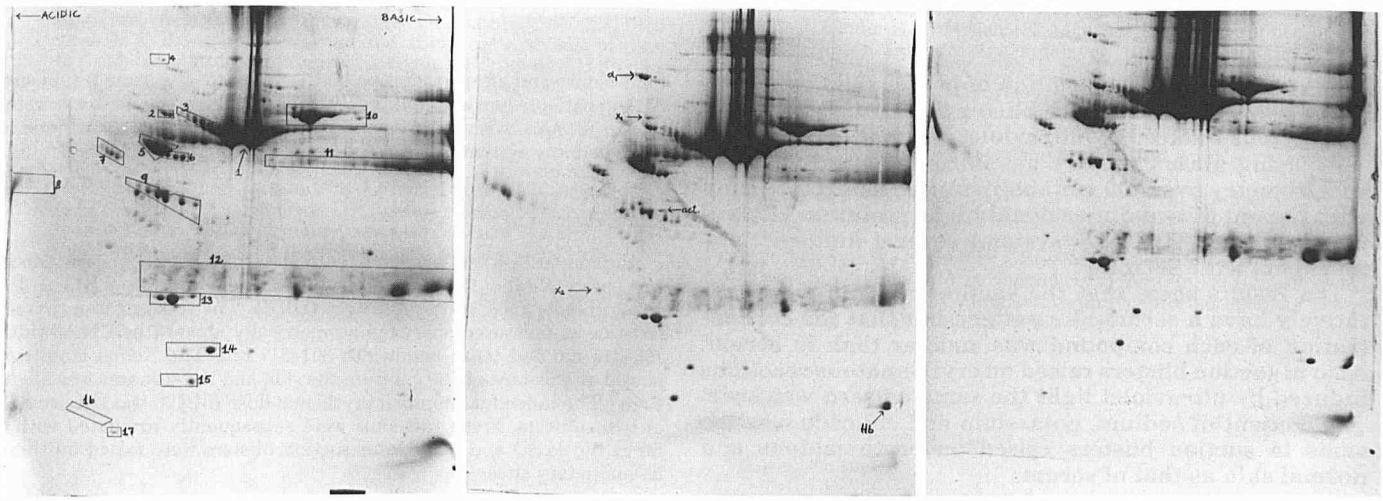

FIG 1. Protein pattern of serum (*left*), blister fluid (*middle*) and blister fluid from UVB irradiated skin (*right*). The proteins were separated by high resolution two-dimensional electrophoresis (ISO-DALT) as described in the text. Some identified protein [10] are labeled: (*1*) albumin; (*2*) α_1 B glycoprotein; (*3*) hemopexin; (*4*) α_1 antitrypsin dimer; (*5*) α_1 antitrypsin; (*6*) Gc-globulin; (*7*) α_2 SH glycoprotein; (*8*) α_1 acid glycoprotein; (*9*) haptoglobin β-chain; (*10*) transferrin; (*11*) IgG γ-chain; (*12*) IgG light chains; (*13*) Apo A-I lipoprotein (HDL); (*14*) haptoglobin α^2-chain; (*15*) prealbumin; (*16*) lipoprotein (LDL); (*17*) Apo A-II lipoprotein (HDL). *Arrows* in the blister fluid pattern indicate spots significantly different from the serum pattern: (α_1) = α_1-antitrypsin dimer; (*Hb*) = hemoglobin β-chain; act. = β and γ-actin, X_1, X_2 = unknown spots.

drates. The procedure also split-triglycerides during the transesterification process and give methyl esters of the released fatty acids. The subsequent silylation procedure converts all unchanged hydroxyl and amino groups into trimethylsilyl (TMS)-derivatives. The resulting TMS/methyl derivatives were finally injected into a combined GC-MS-computer instrument [6]. This consists of a Varian 1400 gas chromatograph, a Varian 112 double-focusing mass spectrometer and a Spectrosystem 100 MS computing system (Varian-MAT, Bremen, G.F.R.). The gas chromatograph was fitted with a variable split injection system and a 25-m glass capillary column (LKB, Stockholm, Sweden), coated with either SE-30 or SP-1000. The number of theoretical plates on these columns was in the range 80,000–90,000. The mass spectrometer was fitted with dual turbomolecular pumps, allowing direct inlet of the effluent from the capillary column into the ion source, which was operated at 70 eV. The instrument was operated in the repetitive scanning mode, and complete mass spectra was recorded every third second and stored in the computer system. After completion of the analysis, mass spectra corresponding to each GC-peak were retrieved from the computer. Comparisons were subsequently carried out between the mass spectra of GC-peaks with identical relative retention times on the chromatograms obtained from serum, blister fluid and blister fluid after UVB-irradiation. In this way it was possible to ascertain identity between every single GC-peak present on all three chromatograms. Furthermore, some of the major peaks were structurally identified by comparison of their mass spectra with a reference file of known spectra [6]. It should be noted, however, that no attempts to identify all GC-peaks were carried out, as the objective of the investigation was to look for *differences* of the metabolite patterns of the various fields.

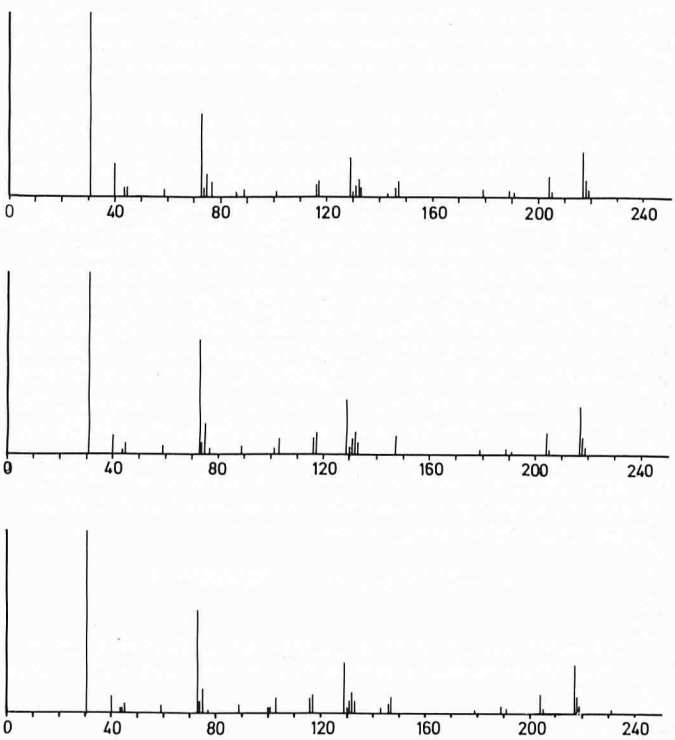

FIG 2. Gas chromatograms of serum (*top*), blister fluid (*middle*), and blister fluid from UVB irradiated skin (*bottom*). The low molecular weight compounds were derivatized with methanol/HCl and silylized with BSTFA before separation of a 25 m SE-30 glass capillary gas chromatography column coupled to a Varian 112 mass spectrometer as described in the text. The temperature was programmed from 80° til 250° at a rate of 6°/min. Mass spectra (over 1000 per chromatogram) were recorded in the repetitive scanning mode.

Determination of the Protein Pattern by Two-Dimensional Electrophoresis (ISO-DALT System)

Serum and blister fluids were analyzed by high resolution two-dimensional gel electrophoresis [7] using the ISO-DALT system developed by Anderson and Anderson [8,9]. The first dimension involved isoelectric focusing in polyacrylamide gel containing 9 M urea, carried out in narrow glass tubes. The second dimension was sodium dodecyl

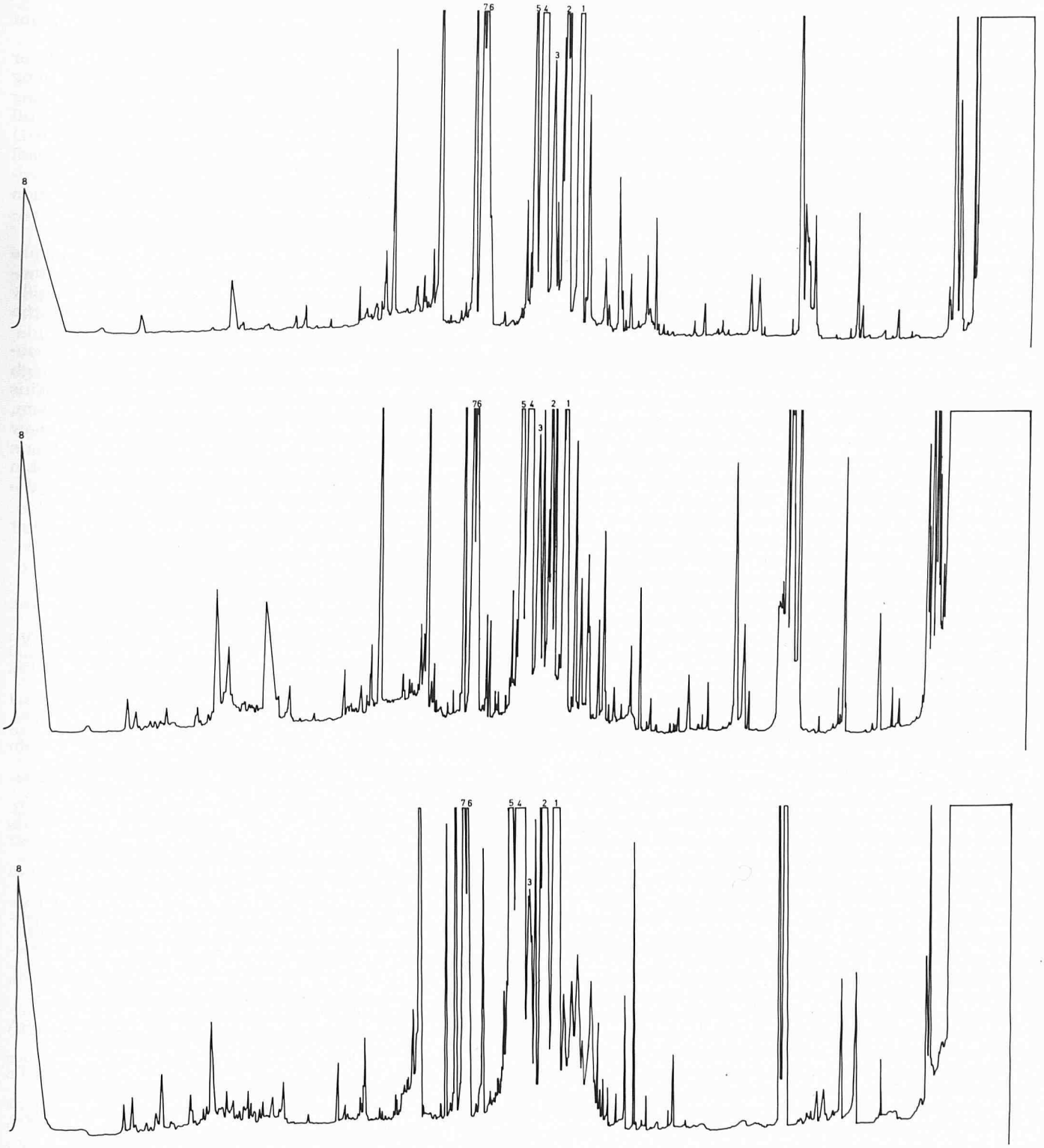

FIG 3. Mass spectra of GC-peak 1, Fig 2 from serum (*top*) blister fluid (*middle*) and blister fluid after UVB-irradiation (*bottom*). Analogous set of results were obtained by comparison of mass spectra from all other corresponding GC-peaks. The absolute identity of some of the GC-peaks were established by comparison with reference spectra. Peak 5 = palmitic acid, peak 6 = oleic acid, peak 7 = stearic acid, peak 8 = cholesterol. The identity of GC-peaks 1–4 is unknown.

sulfate (SDS) electrophoresis in 10%–20% gradient polyacrylamide slab gels. Thus, the complete system separates the proteins according to isoelectric point and molecular weight.

Sample treatment: To 1 part of serum was added 3 parts of 2% SDS/1% dithiotreitol/10% glycerol (and of this mixture 20 μl was used for the two-dimensional separation). Blister fluids were concentrated as their protein content is approximately ¼ of that of serum. 25 μl fluid was dried under N_2, 20 μl SDS/mercaptoethanol/glycerol mixture was added and 15 μl was used for the analysis. After separation the gels were stained in Coomassie Brilliant Blue and photographed.

RESULTS

Blister Formation

The erythema following a UVB dose of 3 times the MED appeared 4–5 hr after the exposure and was maximal at approx-

imately 24 hr. The time for raising bullae was 90 min on normal skin and 70 min on erythematous skin. The blistering sites healed within 6–7 days without producing any visible scarring.

Electrolyte Content of Suction Blisters

The Table shows that the mean values of sodium, potassium and chloride in suction blister fluids were the same as for serum. Also in fluid from blisters raised on erythematous skin provoked by UVB approximately the same values were found.

Multicomponent Analysis

The protein patterns of blister fluids are remarkably similar to the protein pattern of serum from the same person, as determined by the ISO-DALT system (Fig 1). Only minor differences were observed, e.g., the blister fluids (both irradiated and nonirradiated) contained hemoglobin, increased amounts of α_1-antitrypsin dimer (see Fig 1), actin and 2 unknown spots, as compared to the serum.

Figure 2 shows the capillary gas chromatograms obtained from the blister fluids (irradiated and non-irradiated) and from serum. About 1000 mass spectra were recorded during elution of the components. When comparisons were made between mass spectra of corresponding GC-peaks on the 3 chromatograms no qualitative differences were found. An example of this is given in Fig 3 which shows the mass spectrum of GC-peak no. 1, Fig 2 from serum (*top*), from blister fluid (*middle*) and UVB-irradiated blister fluid (*bottom*). As can be seen all 3 mass spectra were identical. The results of the GC-MS investigations therefore show that the patterns of low molecular weight compounds including organic acids, fatty acids, amino acids amines, alcohols and carbohydrates) of the blister fluid and of serum qualitatively are very similar. By comparison of peak areas of corresponding sets of GC-peaks (Fig 2) it can also be concluded that the *relative* amount of each metabolite, with a few exceptions, also appear to be similar in the blister fluid and serum.

DISCUSSION

In the present investigation modern multicomponent analytical techniques have been employed to determine possible differences in the biochemical composition of blister fluids and serum. The gas chromatography—mass spectrometry methods determined over 100 low molecular weight compounds, including organic acids, fatty acids, carbohydrates, aminoacids, etc. The ISO-DALT system determined several hundred protein spots [10] most of which remain to be identified. The results clearly show that the suction blister fluid had a "serum like" pattern qualitatively, although total protein content was about ¼ of the serum level [3,4].

We have previously shown that the blister/serum concentration ratios of total protein, albumin and gamma-globulin were all about ¼ [3]. The difference in molecular weight between albumin (69.000) and gamma-globulin (the major component is IgG with molecular weight 150.000) however, is only 2-fold. Another study using proteins ranging in molecular weight from 6.600 (insulin) to 2.300.000 (low density lipoprotein) demonstrated that the difference in the blister fluid/serum concentration ratio of the proteins well was dependent on molecular weight [13]. The mean total protein content in suction blisters raised on normal and UV-inflamed skin in 7 cases was respectively 20.9 g/l and 26.9 g/l, but the difference was not significant (P value < 0.2) [11].

Separately we have demonstrated that the suction blister procedure *per se* might damage some cells by often finding values for the cytosol enzyme lactate dehydrogenase ranging from 1- to 2-fold the serum value. This marker enzyme was still higher in suction blisters on erythematous skin (P value <0.001) [11]. The same pattern has also been shown for lysosomal enzymes [13]. Most serum enzymes can, however, not be identified with the ISO-DALT system due to too low concentration as is the case with lactate dehydrogenase and lysosomal enzymes.

The presence of hemoglobin in blister fluid does not indicate the presence of red cells since no cells was observed lightmicroscopically. The hemoglobin content in blisters probably stems from red cells in the dermis that is damaged during the suction procedure.

During production of blisters, the fluid has to pass the basement membrane. This study reveals that both low and high molecular weight organic compounds pass this barrier. This also holds true for electrolytes, e.g. the concentration of sodium, potassium and chloride in blister fluid and serum did not show any difference. The same has been found in suction blisters raised on rats [5]. In suction blisters raised on inflamed skin increased potassium values should be expected, but again the same values as for serum were found.

The present investigation therefore strongly suggests that blister fluid is a "filtrate" of serum.

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