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ENERGY METABOLISM OF HUMAN EPIDERMIS DURING PROLONGED SUCTION LEADING TO BLISTER FORMATION

Matti H. A. Härkönen and Väinö K. Hopsu-Havu

From the Departments of Anatomy and Dermatology, University of Turku, and the Department of Clinical Chemistry, University of Helsinki, Finland

Abstract. Concentrations of glycogen, glucose, lactate and ATP in human epidermis were analysed during prolonged suction leading to blister formation as well as in the epidermal roof and fluid of the blister. Epidermal glycogen concentration decreased during suction and increased after blister formation. Lactate concentration increased during suction and decreased after blister formation. Only a minor decrease was recorded in the concentrations of glucose and ATP during suction. The changes were regarded as results of the anoxic compression under the suction device. The time required for blister formation under a constant pressure was found to be shorter in older patients.

Production of subepidermal blisters in human epidermis by suction was first reported in vitro by Blank & Miller (2) and in vivo conditions by Bielicky (1). The latter authors also reported variations in the time of blister formation in patients with different epidermal diseases. He suggested the use of suction blister production as a means for quantitative evaluation of dermal–epidermal adhesion under various experimental and disease conditions. Blister formation in normal skin has since been analysed by several authors (5, 7, 10) by varying the pressure and observing the time required for blister formation as well as applying methods of histochemistry and electronmicroscopy for studies on the blister roof and floor. It was found that within a certain range, higher suction pressure leads to shorter blistering time. The histochemical distribution of PAS-reactive material and activities of several enzymes (succinate dehydrogenase, DPN-diaphorase, TPN-diaphorase, cytochrome oxidase, and acid phosphatase) studied immediately after blister formation were normal. Failure to detect any changes in epidermis when using rather insensitive histochemical methods for the demonstration of enzymes does not, however, need to indicate that any changes in the epidermal metabolism do not take place. We report here findings on the changes in epidermal energy metabolism produced under suction by more sensitive biochemical methods. Changes in the metabolite concentrations of the energy-producing processes appear before any changes occur in the activities of the enzymes involved.

MATERIAL AND METHODS

Normal forearm or, exceptionally, abdominal skin of dermatologic patients, 18–80 years of age, was subjected at room temperature to suction pressure of 200 mmHg for varying periods of time. The time required for the appearance of a vesicle of 3 mm in diameter in two of the five windows of the suction device was registered in 40 consecutive subjects. A number of patients of different ages were selected for the study since later studies included patients of different age groups. Considerably shorter time for blistering was required in subjects suffering from vesicular or bullous diseases, e.g. pemphigus as well as in those who were in an eruptive stage of infective or allergic eczema. These measurements were eliminated from the present material.

In some experiments suction was released before blister formation and the skin was taken for studies by punch biopsy (punch diameter 5 mm). The skin samples were immediately frozen on a cooled copper plate in order to obtain an even epidermal surface. With a cryostat microtome, two sections of 40 μm were cut horizontally from the epidermal surface. Occasional microscopic controls proved that the sections were free from dermal tissue components. Roofs from full-sized (diameter 5 mm) suction blisters were also removed with scissors at different intervals after blister formation, then immediately frozen and weighed. Simultaneously, blister fluid was collected in small tubes, measured and frozen.

The frozen skin samples were stored at -76°C for 1 to 2 weeks before extraction. The samples were weighed

Table I. *Analytical conditions*

The analyses were conducted in a 3 ml fluorimeter tube with 1 ml of reagent to which was added extract equivalent to the amount of epidermis indicated. Abbreviations: G6PDH [Glucose-6-*P*-dehydrogenase (D-glucose-6-phosphate: NADP oxireductase; EC 1.1.1.49)]; PGM [*P*-glucomutase (D-glucose-1.6-diphosphate: D-glucose-1-phosphate phosphototransferase; EC 2.7.5.1.)]; PHRL [glycogen phosphorylase (α-1.4-glucan: orthophosphate glucosyltransferase; EC 2.4.1.1.)]; LDH [heart lactate dehydrogenase (L-lactate: NAD oxidoreductase; EC 1.1.1.27)]; DTT (dithiothreitol, Cleland's reagent, Calbiochem)

Metabolite	Assay conditions	Enzymes	Epidermis used (mg)
Glycogen	50 mM-Imidazole-HCl pH 7.2; 0.01 mM-$NADP^+$; 0.2 mM-5′-AMP; 5 mM-KH_2PO_4; 0.5 mM-$MgCl_2$; 1 mM-EDTA; 0.2 mM-DTT	PHRL, 30 μg/ml; PGM, 7 μg/ml; G6PDH, 0.5 μg/ml	0.5
Glucose	50 mM-tris-HCl, pH 8.0; 0.03 mM-$NADP^+$; 0.3 mM-ATP; 1 mM-$MgCl_2$; 0.2 mM-DTT	HK, 3 μg/ml; G6PDH, 0.5 μg/ml;	0.1
ATP	50 mM-tris-HCl, pH 8.0; 0.01 mM-$NADP^+$; 0.1 mM-Glucose 1 mM-$MgCl_2$; 0.2 mM-DTT	HK, 2 μg/ml G6PDH, 0.5 μg/ml	0.2
Lactate	200 mM-Carbonate buffer pH 9.7; 0.3 mM-NAD^+; 50 mM-Hydrazine	LDH, 37 μg/ml	0.2

on a torsion balance in a cold room at −20°C. Perchloric acid extracts were prepared principally according to Härkönen et al. (4). The samples (1–5 mg) were placed in small glas tubes (6 × 70 mm) containing 10 μl of 0.05 N HCl in 100% methanol in an alcohol dry ice bath at −20°C (50% ethanol). The pieces were crushed and homogenized with nylon rods. Thereafter 100 μl of 0.3 N $HClO_4$ + 1 mM EDTA solution was added at 0°C and after mixing, the suspension was centrifuged in cold. The supernatant (90 μl) was promptly neutralized with a slight (calculated) excess of 2.5 M $KHCO_3$ (12 μl) and $KClO_4$ was removed by centrifugation in cold. The extracts were stored at −70°C until analysed.

Glycogen, glucose and ATP were analysed fluorimetrically in extracts of individual specimens by enzymatic pyridine-nucleotide methods as described by Härkönen et al. (4). The essential details of the methods are summarized in Table I. Since lactate concentration in the skin is high it could be measured directly in 1 ml of reagent. In some cases, when a special adapter with identical narrow (3.2 i.d. × 80) Pyrex tubes was used in a Farrand fluorimeter, the volume of reaction mixture could be reduced to one-tenth of that normally used (1 ml).

RESULTS

Macroscopic observation of the colour and contour of the skin during and after suction revealed signs of tissue anoxia. The skin under the suction device was bleak and compressed, most markedly just around the suction holes. The skin under the open holes was elevated several millimeters towards the vacuum bulb; it was blue-red and congested. After the release of pressure intense erythema persisted for a long time.

The time required for blister formation under the same conditions varied markedly from person to person as can be seen from the scattergram presented in Fig. 1. The same diagram discloses clearly that the blistering time is dependent on the age of the test person, i.e. the blister was formed considerably faster in older patients than in younger ones. The time required for blister formation in patients of 20 years was roughly twice that required in persons above 60 years of age.

Normal concentrations of glycogen, glucose, ATP and lactate in the intact epidermis are given in Table II.

Epidermal samples taken at different time intervals after the application of suction revealed that glycogen decreased to about half of the original concentration (Fig. 2). The decrease was especially marked in the samples taken from 2 patients recovering from eczema. They also had a much higher initial glycogen concentration (average 7.80 nm/kg) than the other patients. Glucose concentration remained essentially unchanged (Fig. 2). There was some tendency to a decrease of ATP after 2 to 3 hours. Lactate increased about 50% during the observation time (Fig. 2).

After suction blister formation the concentrations of the same substrates in epidermis as well

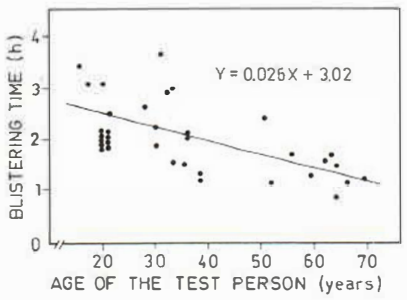

Fig. 1. Time required for blister formation as a function of the age of the test person.

as in blister fluid were followed. In blister roof, glycogen increased to about half of the normal epidermal value (Fig. 2). Glucose and ATP concentrations did not show any clear changes in blister roof (Fig. 2). In blister fluid, glucose concentration was close to that of plasma and did not alter much during 6 hours (Fig. 3). Lactate concentration in epidermis tended to decrease towards the normal level (Fig. 2) and in blister fluid a slight increase was observed (Fig. 3). Additionally, the volume of blister fluid was found to decrease and the protein concentration to increase during the period after the release of suction. Immediately after the release of pressure, protein concentration was between 1.8 and 2.4% while 6 hours later the concentration was 2.0–4.5%.

DISCUSSION

These data reveal that the adhesion of the epidermis to the dermis, i.e. to the basement membrane, is age-dependent. It remains to be seen whether age-induced changes in "viscosity" of the tissue fluid or changes in the morphological fibre

Table II. *The concentration of four metabolites in epidermis, expressed as mmol/kg wet weight*

The values of own findings are the means (±S.E.) for five specimens except in glycogen concentration, where four samples were analysed. Glycogen is expressed as glucosyl units

Metabolite	Own finding	Halprin & Ohkawara 1965 and 1966
Glycogen	3.28±0.50	2.6
Glucose	3.47±0.45	1.25
ATP	1.34±0.22	0.28
Lactate	12.36±1.23	7.05

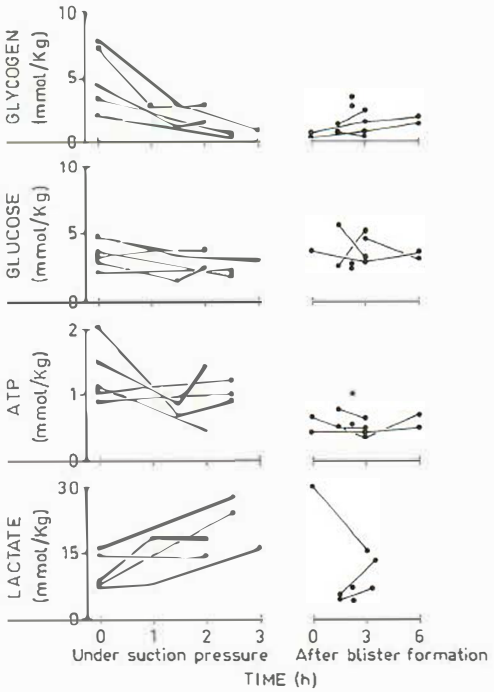

Fig. 2. The concentration of glycogen, glucose, ATP and lactate in the epidermis during a prolonged suction and after blister formation.

structures of the semidesmosomes are the source of the weakened cellular adhesion which leads to a more rapid blister formation in the skin of older persons. A considerably shorter time was required for blister formation in patients suffering from vesicular dermatoses which is in agreement with earlier observations (1).

The increase in the protein concentration of the blister fluid after the release of suction pressure is explained by the reabsorption of the fluid. Six hours after the release of pressure, the volume of the blister fluid was markedly smaller than the

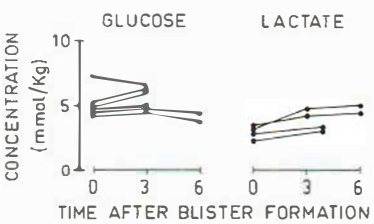

Fig. 3. The concentration of glucose and lactate in the suction blister fluid at different times after blister formation.

original value. Reabsorption of the proteins may not proceed equally to water and electrolytes and thus an increase in the protein concentration is found to take place.

Among the metabolites studied in this work only glycogen can be demonstrated histochemically. But glycogen is hardly found in the normal adult epidermis whereas it is present during the foetal period. Histochemically demonstrable epidermal glycogen appears during inflammation or abnormal proliferation. A transient appearance of glycogen follows after a minor epidermal trauma (6). In accordance with this, we found glycogen values in the post-eczema skin more than twice those in the normal epidermis. Comparison of the normal metabolite values with those reported by Halprin & Ohkawara (3) reveal our values generally somewhat higher. This may partly be due to the fact that epidermis studied by us was not contaminated by dermal tissue, which is known to be metabolically considerably less active than epidermis. Another reason may be the difference in the methods used by us and by Halprin & Ohkawara (3).

The macroscopic observations of the skin suggest that the epidermis under suction was deprived of a contact to dermal fluid circulation and thus the transport of metabolites and oxygen to and from the epidermis was markedly impaired. As soon as the blisters start to form, the metabolites of the epidermis can be released into blister fluid and, on the other hand, the metabolites from the blister fluid can enter the epidermis. It is to be expected that such changes in the fluid circulation to and from the supporting dermis during several hours of suction produce changes in the metabolite levels of the epidermis.

The most remarkable changes in the substrates of energy metabolism during suction took place in the concentration of glycogen and lactate. Concentration of glycogen fell to one-half and that of lactate rose roughly to doubled values. A decrease in the glycogen concentration would mean that, under suction pressure, the synthesis of glycogen was lower than the glycogen breakdown. This could be caused either by a decrease in the rate of synthesis or by an increase in the rate of breakdown. Increased lactate concentration would suggest the latter mechanism. Since glucose and ATP concentrations at the same time decreased very little, even although the inflow of glucose most probably was markedly decreased, it appears that energy in epidermis was primarily obtained from glycogen.

After release of suction, a slow increase of glycogen seems to take place in the epidermis. This might be due to a markedly enhanced glucose supply: the glucose concentration in the blister fluid was nearly twice as high as in the epidermis. This is in keeping with the earlier observation that the glucose concentration in the epidermis is about 55 % of that in the plasma (8). This is also in agreement with our observation that glucose concentration in the epidermis during the last hours of pressure is lower than soon after blister formation.

Another change which occurred in the epidermis soon after blister formation was the marked decrease in lactate concentration. This would mean a freer diffusion of lactate from epidermis to the blister fluid than to the dermis under suction congestion. Since lactate concentration in normal human serum is 0.7–1.8 mmol/l (in our clinics), there is a clear concentration gradient from epidermis to the blister fluid and from there to blood plasma.

These findings prove that the energy-producing metabolism is changed in the epidermis during suction blister formation. It is quite possible that the changes in the basal cell layer are even more marked than are shown in studies dealing with whole-thickness epidermis. It remains to be seen whether the shorter suction times for blister formation in older persons are indicative of lower concentrations of metabolites and enzymes active in energy-producing metabolism in the epidermis at old age (9).

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Väinö. K. Hopsu-Havu, M.D.
Skin Biology Research Unit
Department of Anatomy
University of Turku
Turku 3
Finland