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MICROCALORIMETRIC STUDIES OF HUMAN SKIN CELLS

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ABSTRACT

A short but comprehensive review is given about direct calorimetric measurements on human skin cells in vitro.

INTRODUCTION

Since several years microcalorimeters have been used as an analytical technique on cellular systems (ref.1) but only occasionally on tissue cells (ref.2). Direct calorimetric measurements of the heat production characterizes the gross calorific output reflecting metabolic events within the biological specimen as well as physical interactions with the ambient milieu. Though the sources and the magnitudes of the heat production are mostly unknown in detail the heat signal still gives strong indications of the overall metabolic intensity and of the cellular response to metabolic regulators.

In this way calorimetry presents itself as an advantageous assay method not at least because it is a non-destructive technique and cells need not be sophisticatedly prepared.

Skin structure

The human skin is composed of three anatomically distinct layers. From the surface downward there are the epidermis, the dermis and the subcutis.

The main cell type of the subcutis is the lipocyte or adipocyte which produce lipids in their cytoplasm and form the subcutaneous fat.

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The dermis or corium (thickness 1-4 mm) is mostly composed of dense, fibroelastic tissue within which are embedded blood vessels, muscles, nerves and eccrine units. The predominant cell type is the fibroblast, which is the builder cell of the connective tissue. Fibroblasts are metabolically active cells.

The epidermis (average thickness 40-140 µm) is a stratified, squamous epithelium of a high metabolic rate. It is populated mostly by keratinocytes. The replication of keratinocytes occur at the basal layer of the epidermis, then they migrate upwards and differentiate toward maturation. The transfer takes about 4 weeks and the keratinocytes undergo characteristic changes in shape and morphology, i.e. they dehydrate and form the horny layer.

On the whole the skin serves as the outer body shell and represents by far the largest (at least 10% of the body weight) and most active organ of a mammal. Diagnostic and therapeutic measures very often start with the skin because symptomatic and metabolic changes of the skin reflect disturbances of other domains of the body.

RESULTS

Skin Biopsies

The earliest calorimetric measurements on human skin were done on peasized skin biopsies comprising epidermis, corium and subcutis (ref.3, ref.4). The aim of these investigations was to point out a correlation between the thermal effects going along with allergic reactions and the basal metabolism of skin. The hormone histamine released from mastcells during allergic conditions was thought to be responsible for an increased metabolic activity in the skin tissue. In fact, after addition of histamine or other inflammatory substances to healthy skin or after degranulation of mastcells in normal skin and in mastcell tumours, exothermic effects were found in good accordance with increased rates of respiration. With these experiments compact pieces of about 50 mg wet weight were used in batch - and sorption-microcalorimeters. Unfortunately no exact figures were given, but a rough estimation of the calorimetric data yields approximately a heat production rate of 1 mW per g of dry skin, which corresponds satisfactorily with a value of 1.3 mW derived from oxygen consumption rates (ref. 5).

Adipocytes

Since the subcutaneous fat is not intensively involved in the biological and biochemical function of the skin, the calorimetric values shall be given here only for completeness (ref. 6, ref. 7). Most of the experiments were done with suspension of a few hundred-thousands of adipocytes. Unstimulated adipocytes exhibit not a very active metabolism, and the recorded heat output is very low compared with other skin cells (Table). Results from adipocytes are of value in studies on the energy expenditure of whole organisms or on obesity of humans.

Epidermal slices

Up to 1976 experiments on the total metabolism of whole skin or different layers of it were performed manometrically or enzymatically. The first calorimetric measurements on energy metabolism were reported by Anders et al. (ref. 8), who investigated the heat output of small pieces of epidermis under different metabolic conditions (Table). Lateron the experiments were extended to investigations of the enzymatic activity of lactate dehydrogenase LDH (ref. 9, ref. 10) a key enzyme in glycolysis, and to investigations of UV-irradiated skin (ref. 11). With all these experiments the epidermis was sheared off from the cutis and small pieces (1-2 cm^2, 5-10 mg fresh weight) were stretched in frames and placed into the vessel of a batch-calorimeter- Substrates could be added to the reaction vessel through a syringe. The disadvantage with compact slices of skin is a considerable portion of damaged cells within the specimen due to the separation procedure leading to uncontrollable conditions (cell lysis, leaking enzymes).

Fibroblasts

Skin cells taken from the original tissue can be subcultured over several passages without loosing their original properties. Such a primary culture can be kept alive as a confluent layer anchored on a solid matrix. Only cells from a transformed, established cell line may be cultured in suspension. To meet the more natural conditions for fibroblasts a foil technique was described (ref. 12), by which cells in a monolayer culture could be measured within a calorimeter.

TABLE

Heat production rates of different tissues of human skin

Type of Skin Cell	Heat Production Rate			Reference
	Cellular (pW/cell)	Weight Specific (mW/g)		
Skin biopsies	-	1	dry weight	estimated from 4
Epidermal slices				
endogen	0.5	5.8	dry weight	
aerobic	3.3	34		8,9,10
anaerobic	1.9	19		
Adipocytes in suspension	19	0.13	lipid weight	7
Fibroblasts in monolayer	40	60	dry weight	12
Keratinocytes in monolayer				
normal cells	83	207	protein weight	16
transformed cells	134	314		

Outside the calorimeter fibroblasts were seeded on a STERILIN™ plastic-foil and after attachment of the cells the foil was introduced into the calorimetric vessel. By this method the growth of the cells as well as the heat output during confluency could be determined. A cell number of 1 to 5 million was necessary to get reproducible results. By means of that foil-technique the influence of dermatologically active drugs on the general metabolism of fibroblasts could be screened (ref. 13, ref. 14, ref. 15).

Keratinocytes

Keratinocytes cultivated on glas or plastic surfaces do not develop the complete, terminal pattern of differentiation as found in the living skin. The in vivo conditions for keratinocyte growth require the contact with the air and a nutrition supply through the basal layer. The foil-technique used with fibroblasts was therefore improved (ref. 16) by taking PETRIPERM™ tissue culture

dishes. The bottom of the dish was coated with acid soluble collagen which serves as an anchorage matrix for the cells, and, furthermore, the hydrophilic material of the bottom allows oxygen penetration through the PETRIPERM™-membrane. After pre-cultivation of the keratinocytes the membrane could easily be removed and transferred into the vessel of a batch-microcalorimeter. This technique requires about half a million cells and led to reproducible heat production profiles. It can be easily adapted to calorimetric studies of other mammalian cells in vitro.

CONCLUSION

The high sensitivity of modern microcalorimeters of about 1 μW and progress in cell culturing techniques made calorimetry available during the last decade for the study of human skin cells in vitro, whether in compact pieces or in cell cultures. Thus data on the basic energy metabolism were available (Table). But, furthermore, calorimetry could be applied in screening topically active, therapeutic agents and attempts are in progress to develop a test procedure for healthy and diseased skin.

Note

Reports on thermal analysis (DTA, DSC) of skin specimen as well as experiments using the different methods of thermology in situ were not included in the present review. These methods are of value in studying the structure of material (e.g. lipid content, water content) or they reveal conditions of the cutaneous blood flow and the cutaneous thermal conductance.

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