To sum up, whatever the ambient temperature and related body temperature, edible dormice obviously lack an endogenous circannual mechanism basically responsible for the parallel-running annual reproductive and thyroid cycles observed in nature. This species therefore clearly differs from other hibernators capable of developing annual reproductive cycles in a constantly cold environment, such as the European hedgehog[9], or garden dormouse[10] or in a constantly warm one (e.g. ground squirrels and chipmunks[4,21].

In fact, in the edible dormouse, no functional activity has so far been shown to display a cycle with an endogenous circannual pattern. Consequently, the annual testosterone and thyroxine cycles observed for this species in its natural environment appear to depend essentially on the annual temperature cycle of that environment[14].

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Heat evolution of cultured human keratinocytes

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Summary. The heat production of normal and transformed human epidermal keratinocytes precultured in Petriperm™ tissue culture dishes was measured calorimetrically. For this purpose, the membrane at the bottom of the culture dish was cut out aseptically and put into a microcalorimeter vessel with the cell layer inwards. A continuous heat output of (83 ± 12) pW/cell was measured for normal keratinocytes from a confluent primary culture. A value of (134 ± 35) pW/cell was obtained when the transformed keratinocyte line SV-K14 was used. The method described in this paper is simple, leads to reproducible results, and can be easily adapted to the calorimetric study of other mammalian cells in vitro.
Key words. Microcalorimetry; heat production; keratinocytes; monolayer culture.

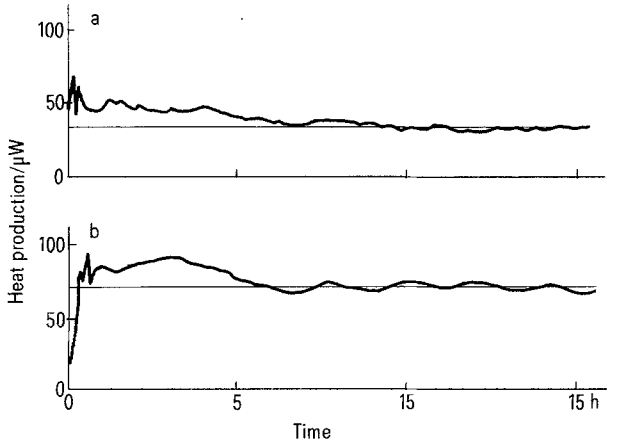
Microcalorimetry is a universal tool to study thermal changes. The high sensitivity (1 μW) of modern microcalorimeters and progress in cell culture techniques make this tool available even for the study of anchorage dependent mammalian cells in monolayer culture which, until now, have been difficult to investigate. Two main techniques have so far been developed using either microcarriers[1] or tissue culture plastic foils[2]. The first technique suffers from the difficulty of controlling cell growth and morphology by microscopic inspection, whereas the second is intricate unless a special frame to support the foil is constructed.

In this paper, we describe improvements in the second technique, making use of a new entry in the field of monolayer culture, the Petriperm™ tissue culture dish. The bottom of this dish consists of a flexible membrane to which the cells adhere during precultivation. The membrane can easily be removed and transferred into the microcalorimeter where the cells, in the presence of an appropriate culture medium, continue to grow and produce a well-measurable and reproducible heat signal.

Material and methods. Tissue culture dishes. Petriperm™ tissue culture dishes with a hydrophilic membrane of 5 cm diameter as the cellular support were obtained from Heraeus France, Orsay. In order to facilitate the attachment and growth of normal keratinocytes they were coated with acid-soluble collagen (Sigma Type III) according to the procedure of Liu and Karasek[3]. Collagen coating was not necessary when transformed keratinocytes were used.

Organisms and preculture conditions. Normal keratinocytes. Human breast skin from plastic surgery was used as the source of normal keratinocytes. Thin split-thickness skin sections were obtained by means of a Castroviejo keratotome adjusted to a depth of 0.3 mm. Epidermis and dermis were separated after treatment with 0.3% (w/v) trypsin (Gibco, 1:250) for about 1 h at 37 °C as described by Liu and Karasek[3]. Dermal and epidermal flaps were agitated gently in growth medium to liberate basal and malpighian cells which were collected by centrifugation at 800 × g for 5 min. The cells were seeded at a density of 10^5 translucent cells per cm^2 on collagen coated Petriperm™ dishes. After 2 h cell debris and non-attached cells were removed by aspirating the medium. The attached cells were grown at 35.5 °C in a humidified incubator (5% CO_2) to confluency using Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal calf serum, 1 μM hydrocortisone, 100,000 units/l penicillin and 100 mg/l streptomycin.

Transformed keratinocytes. The SV-40 transformed human foreskin keratinocyte line SV-K14 (a kind gift of Dr B. Lane, Imperial Cancer Research Fund, London) was used after about 20 passages. The cells were grown in non-treated Petriperm™ dishes to confluency at 35.5 °C (5% CO_2, humidified atmos-



Power-time curve (thermogram) of human epidermal keratinocytes in a primary culture of normal cells with a final cell number of 4.2×10^5 (a) and in a culture of the transformed line SV-K14 with a final cell number of 5.5×10^5 (b).

phere) in a 1:1 mixture of DMEM and Ham's medium F12 containing 100,000 units penicillin, 100 mg streptomycin, and 250 µg amphotericin B per l of the medium which was supplemented with 5–10% fetal calf serum depending on the batch.

Preparation of the cultures for calorimetric experiments. After aspirating the culture medium the Petriperm™ dishes were placed upside-down under a laminar flow hood. The outer side of the bottom membrane was sterilized by the addition of absolute ethanol which within 1 min was completely removed by aspiration. Then the membrane was cut out with a sterile scalpel and placed aseptically with the cellular side inwards in a heat-sterilized calorimeter vessel made of stainless steel (17 mm diameter, 80 mm height). After slowly adding 10 ml DMEM (in which the sodium bicarbonate was replaced by 25 mM HEPES, pH 7.2), the calorimeter vessel was closed tightly, prethermostated at 35.5 °C for 15 min and introduced into a differential batch calorimeter (Bioflux from Thermanalyse, France). The reference vessel was treated in the same manner but charged with a blank Petriperm™ membrane. The calorimetric system was allowed to equilibrate for 20 min. Then the heat production rate was continuously recorded as a power-time curve (thermogram) at a sensitivity of 43.7 µV/mW. At the end of the experiments the Petriperm™ membranes were inspected microscopically.

Protein determination. The cells were extracted with 4 ml 5% (w/v) trichloroacetic acid and then 'solubilized' in 4 ml 0.5 N NaOH. To 100 µl of these solutions, 700 µl 0.071 N HCl and 200 µl Bio-Rad Protein Assay Dye Reagent (Bio-Rad Laboratories, München, FRG) were added. The samples were processed for protein determination as described in the protocol supplied with the reagent. Bovine serum albumin, fraction V, was used as the standard.

Results and discussion. The keratinocyte is the principal cell type of the upper layer of the skin, the epidermis. Its replication takes place in the basal cell layer from which the cells move upwards, undergoing a distinct program of terminal differentiation leading to the dead horny layer. The time of transfer for one cell through the entire epidermis is about one month.

Normal keratinocytes in primary culture preserve most of their in vivo properties although their differentiation program is incomplete[4]. Confluent cultures consist of differentiating cells and those with basal cell character. Their proliferative activity, however, is very low as can be deduced from the few mitotic figures to be observed under the inverted phase-contrast microscope. The heat profile of such a culture is given in the figure (a). The fluctuations in the first hour are due to physical pertubations and thermal equalization. Within the next five hours, the power-time curve declines to a rather constant level representing an equilibrated energy metabolism. The increased heat production observed in the first phase of the experiment can be explained by enhanced metabolic activity due to the medium change and to the repair and replacement of cells which have been damaged by the transfer of the Petriperm™ membrane into the calorimeter vessel. From the plateau values of nine independent experiments a heat evolution (± SD) of (83 ± 12) pW/cell or (207 ± 30) mW/g protein has been calculated which is of the same order of magnitude as the values reported for other tissue cells[2,5,6].

Transformed cells are 'immortalized' and keep their proliferative capacity[7]. Even under confluent conditions a large number of mitotic figures can be seen. The surplus of cells detaches from the support and dies. The higher metabolic activity of the transformed cells is reflected in the higher heat output of (134 ± 35) pW/cell or (334 ± 87) mW/g protein (fig., b) which has been calculated from 11 independent experiments.

In conclusion, the described procedure using Petriperm™ tissue culture dishes provides a simple means for the calorimetric study of transformed and nontransformed anchorage dependent cells.

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On the quaternary structure of *Kinixys erosa* hemoglobins

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Summary. Kinixys erosa hemoglobins TH_I and TH_{II} comigrate on SDS-PAGE with mol. wt of 18 kD. Consistent with their amino acid compositions, trypsinolysis of the succinylated molecules and hydrolysis of cyanylated TH_{II} yielded five and two fragments respectively.

Key words. Chelonian hemoglobins; *Kinixys erosa;* quaternary structure; homomerism.

In earlier publications[1,2] the isolation and determination of some physicochemical properties of electrophoretically homogeneous hemoglobin fractions from red cell hemolysates of the testudinid *Kinixys erosa* were described.