The complex relation between olfaction and the hypothalamo-pituitary-adrenal axis is underlined. Even if the three olfactory pathways seem to be involved, the anterior branch of the anterior commissure might play a preponderant role in an inhibitory olfactory control of the hypothalamo-pituitary-adrenal axis in the intact rat. However, the origin of the corticosterone level increases after an olfactory lesion remains unknown; it may depend upon several factors which have yet to be studied.

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Suppression of annual plasma testosterone and thyroxine cycles in the edible dormouse *Glis glis* under constant photoperiod at 24 °C

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Summary. Exposure of male edible dormice all year round to an unvarying photoperiod and warm temperature disrupted their biological cycles; hibernation was almost completely suppressed, and short lived infradian cycles of body weight, and of plasma testosterone and thyroxine were measured instead of the normal annual pattern.
Key words. Hibernation; annual cycles; reproduction; thyroid.

Hibernators have long served as experimental models for the exploration of thermoregulatory mechanisms[1]. In addition, several species of hibernating mammals have been found very suitable for studies of the mechanisms underlying annual biological cycles. Most of the species so far studied in this respect have been shown to maintain endogenous circannual cycles of hibernation and body weight under a constant photoperiod (12 h light and 12 h dark) and cold temperature. They include the European hedgehog *Erinaceus europaeus*[2], the woodchuck *Marmota monax*[3], the chipmunks *Eutamias* and *Tamias striatus*[4], the golden mantled squirrel *Citellus citellus*[5], and other ground squirrels[6], the European hamster *Cricetus cricetus*[7] and the meadow jumping mouse *Zapus hudsonius*[8]. Under similar conditions, European hedgehogs[9] and the garden dormouse *Eliomys quercinus*[10] also displayed circannual cycles for testosterone levels. On the other hand the edible dormouse *Glis glis* exhibited a different regulatory pattern since its response to a constant cold environment and an unvarying photoperiod was the disappearance of annual rhythmicity, but persisting infradian cycles comprising periods of a few weeks or months observed for hibernation and body weight[11-13] and for plasma testosterone and thyroxine[14].

Since edible dormice also displayed the special characteristic of requiring normothermia as a prerequisite for any increase in their plasma testosterone and thyroxine titers[15], the present experiment was designed to explore the annual pattern of both these hormonal secretions, when animals were kept in a warm instead of a cold environment.

Material and methods. Eleven adult male edible dormice were kept from November 1982 through November 1983 in a room equipped with an automatic device providing a photoperiod of 12 h light (100 lux) and 12 h darkness; the temperature was maintained at 24 ± 0.2 °C. Animals were housed singly in wire mesh cages (65 × 34 × 30 cm) containing a nest-box furnished with straw and an automatic device providing drinking water. They had free access to a standard hamster chow (Provimi, Paris) and were given a few apples twice a week. Individual wake/dormancy behavior was checked daily. In addition, on a fixed date and hour each month, animals were weighed and their rectal temperature quickly measured with a Digi-sense thermometer; 1 ml blood samples were collected by heart puncture, centrifuged and stored at −30 °C. Temperature measurement, heart puncture and blood sampling were performed under fluothane anesthesia and lasted less than 2 min. Plasma testosterone was measured with RIA kits from the Commissariat à l'Energie Atomique (Saclay, France), and thyroxine levels were assayed by the radio-competition method as in earlier experiments[14,15]. Control dormice were kept under natural temperature conditions and a 12 h light/12 h dark schedule. Results are expressed as means ±SEM. For statistical comparisons, both within this experimental group and with earlier experiments, we used Student's t-tests and one-way ANOVA followed by Fisher's or

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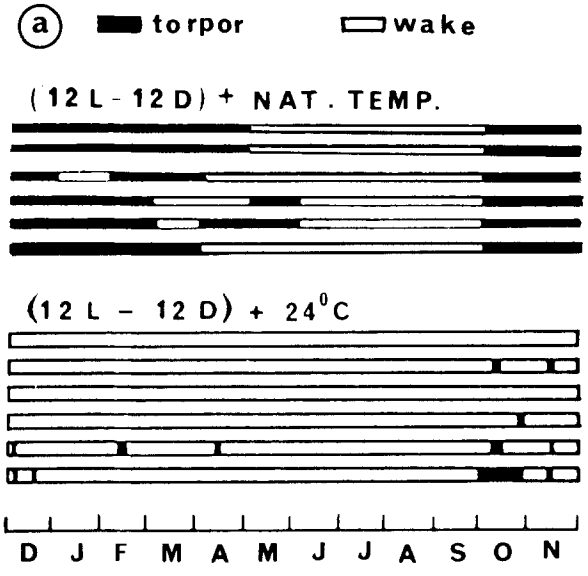

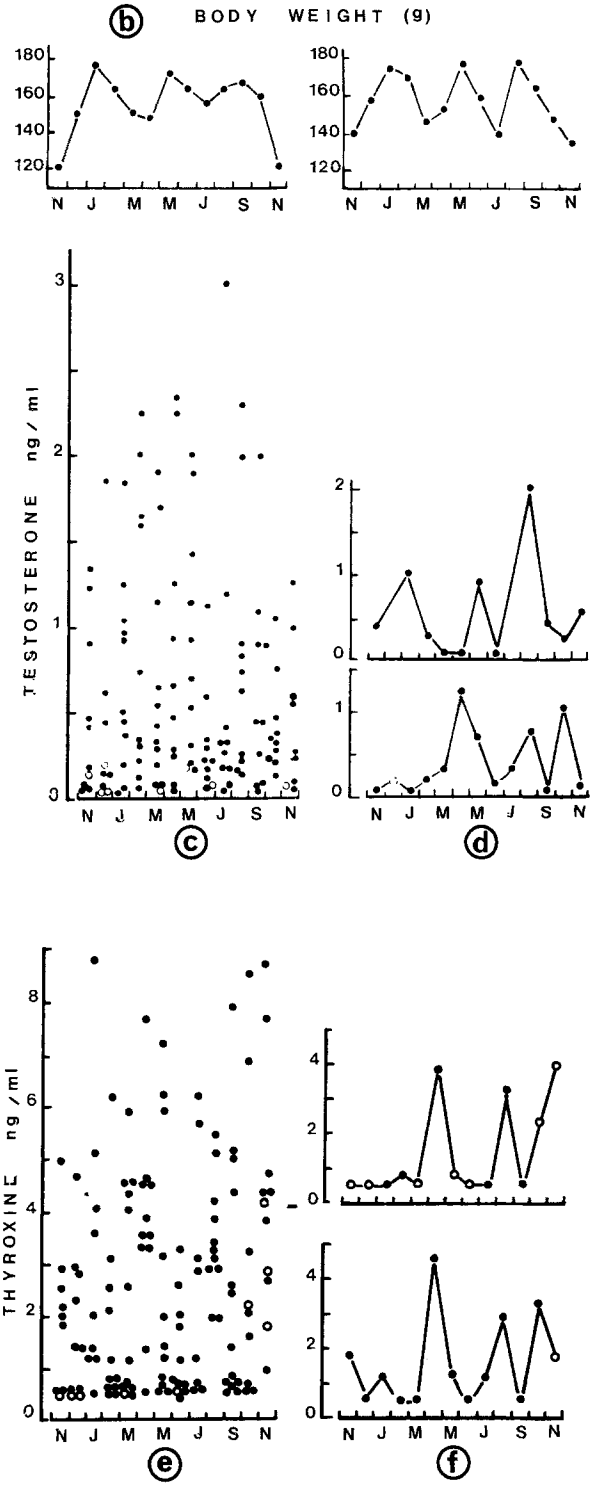

Suppressive effect of constant warm temperature on annual biological cycles in edible dormouse.
(a) Loss of hibernation cycle: Hibernating patterns of representative specimens under a constant photoperiod of 12L–12D, either in the natural temperatures (top; n = 6), or under a constant temperature of 24 °C (bottom; n = 6). (b–f) Loss of annual cycles in body weight and levels of plasma testosterone and thyroxine in dormice under a constant photoperiod of 12L–12D and a constant temperature of 24 °C. ●: active, and ○: lethargic state at measurement times. (b) Two individual patterns for body weight cycles measured all the year round, (c) Annual distribution of plasma testosterone levels measured all the year round, and (d) Individual patterns for infradian plasma testosterone cycles. (e) Annual distribution of plasma thyroxine levels measured all year round, and (f) Individual patterns for infradian plasma thyroxine cycles.

Wilcoxon's test depending on data distribution, and Kendall's tau test to evaluate specific correlations.

Results and discussion. It is generally accepted that for most species, entrance into seasonal dormancy requires a low ambient temperature[16], although a few species were shown to display phases of torpor even in a constantly warm environment. Of these, ground squirrels continued to exhibit a hibernating behavior at 20 °C (*Citellus lateralis*[17]) or 30 °C (*Citellus citellus*[18]), the meadow jumping mouse *Zapus hudsonius*, at 20 °C[8], and a few species of tropical and European bats, at 28 °C[19]. Clearly, the behavior of edible dormice kept in a warm environment was closer to the usual behavior, as the present series displayed an essentially active normothermic life pattern (fig., a). A few animals exhibited occasional short-lived bouts of relative torpor, with body temperature falling to 20–25 °C. However, these phases of mild lethargy never exceeded a few days, and the mean annual number of torpid days amounted to about one seventh of that observed in the controls kept at natural temperature. Maintenance of animals in a warm room suppressed the annual body weight cycle observed under natural conditions[15] (fig., b). This cycle was replaced by shorter infradian fluctuations, as noted earlier when edible dormice were kept in a warm[20] or cold environment[14]. Similarly, no annual cycles were detected for testosterone or thyroxine, and lower or higher titers for these hormones were randomly distributed throughout the year (fig., c and e). However, closer observation of individual patterns revealed that both hormones fluctuated according to an infradian pattern fairly similar to that noted in earlier experiments in a cold environment (fig., d and f). On the other hand, mean testosterone levels were significantly higher than in the earlier cold room experiment (0.65 ± 0.06 vs 0.45 ± 0.06 ng/ml) ($p < 0.04$), whereas the opposite was the case for plasma thyroxine, which was depressed in the warm room experiment (2.6 ± 0.2 vs 6.2 ± 0.2 ng/ml) ($p < 0.001$). Despite this difference, it is interesting to note that the infradian cycles of testosterone and thyroxine, assessed by Kendall's tau test, were significantly correlated ($p < 0.05$), and all the more interesting as similar close correlations were earlier shown to link the annual testosterone and thyroxine cycles prevailing in the natural environment ($p < 0.01$) with the same test[15].

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-