COTTON en ont émis l'hypothèse[10], et lierait ainsi le déroulement de la vitellogenèse à celui de la mue chez *O. gammarella*.

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*Summary.* Ecdysterone, administered to *Orchestia gammarella* females (200 µg/g), has no positive effect on the female-specific protein synthesis and vitellogenesis.

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## The Effect of Heat on Rat Pineal Hydroxyindole-*O*-Methyl Transferase Activity

Exposure to continuous light was found to affect basal and specific metabolic pathways in the rat pineal gland, causing inhibition of most processes and enhancing others[1]. Environmental stimuli other than light, e.g. temperature[2,3] and noise[4], have also been found to affect pineal metabolism. Exposure of rats to low temperature produced pineal hypertrophy and increased metabolic activity[2], while heat decreased pineal contents of protein and RNA[3]. The possibility was considered that the pineal gland could, in addition to the stimuli of light, also be transducing stimuli of temperature[3,5]. In order to give further substance to this postulation, it was decided to investigate whether exposure to continuously elevated environmental temperature affects specific pineal metabolic processes. The effect of heat on the terminal reaction in the synthesis of melatonin, the methoxylation of N-acetyl-serotonin by hydroxyindole-*O*-methyl transferase (HIOMT) is reported here.

*Materials and methods.* Male rats weighing 160–180 g were divided into 3 groups of 8 animals each (4 to a cage). 2 groups were exposed to constant heat of 33 ± 1 °C for 1 and 3 days respectively, and the 3rd group, which served as control, was kept at a temperature of 23 ± 1 °C. Light was provided by overhead fluorescent tubes which were automatically switched on at 06.00 h and off at 18.00 h each day. After 1 or 3 days' exposure to heat, the rats were decapitated between 22.30 h and 23.30 h, their pineal glands were removed rapidly, rinsed in cold phosphate buffer and ground in 0.5 ml phosphate buffer pH 7.9. All samples were made in duplicate. HIOMT activity was estimated immediately by the method of AXELROD et al.[6]. N-acetylserotonin used was purchased from Sigma Chemical Company and $^{14}$C-methyl-S-adenosylmethionin (spec. activity 58 mCi/mmole) from New England Nuclear.

*Results.* From the Table it can be seen that already after one day's exposure to heat of 33 ± 1 °C a significant decrease (17%) in pineal HIOMT is evident. When exposure is prolonged to 3 days, the depressant effect of heat on HIOMT increased still further to 26.5%.

Effect of continuous heat (33 ± 1 °C) on rat pineal HIOMT activity[a]

| | Days of exposure | | Control |
|---|---|---|---|
| | 1 | 3 | 23 ± 1 °C |
| | 46.1 ± 2.4[b] | 40.8 ± 2.8[c] | 55.4 ± 2.7 |
| Number of samples | 16 | 14 | 23 |

[a] µµmole melatonin $^{14}$C formed/pineal/h ± S.E.M. [b] $p < 0.001$; [c] $p < 0.0005$.

*Discussion.* Exposure of adult male rats to continuously elevated temperature of 32–34 °C caused a significant decrease in the melatonin forming capacity of the pineal gland. The effect was already evident after 24 h exposure to heat, and increased still further during the course of the next 48 h (Table). Examination after 10 days' exposure did not reveal any further changes. Comparing this effect to the diminished pineal contents of RNA and protein, which were observed only after rats had been exposed to the same temperature (33 ± 1 °C) for 20 and 30 days[3], one may assume that the effect of heat on the melatonin-forming enzyme HIOMT is not a result of a general inhibition of metabolism but rather a specific response to heat.

Exposure of rats to extreme temperatures has converse effects on pineal gland and gondas: low temperatures (3–10 °C) enhance the metabolic activity of the pineal gland[2,5] but cause involution and decreased weight of gonads[7,8]. In the present study, it was found that an elevated temperature of 33 ± 1 °C caused a decrease in the HIOMT activity involved in the specific metabolic process of production of melatonin, considered an active pineal hormone. This is in agreement with previous findings on general protein metabolism[3]. Consequently, because of the decreased pineal metabolism brought about by heat, a concomitant increase in the development of the gonads may occur through activation of the neuroendocrine axis, as indeed has been reported[9]. Heat thus acts similarly to light, which produces an even more marked decline in the activity of HIOMT in rats maintained in continuous light[10].

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It is interesting to compare the effects of heat and light on the peripheral glands in relation to pineal function. Regarding photic stimuli, it was demonstrated that pineal active hormones abolish most effects exerted by lhigt, and vice versa, pinealectomy erases the effects of darkness on the peripheral glands[11]. As to temperature stimuli, such a relationship appears less likely[8]. Both heat and light have a depressant effect on pineal metabolism, though they may act through different mechanisms.

It has been noted that in 12-day-old suckling rats which are poikilothermic, brief single exposures to temperatures of 7 and 34 °C failed to alter pineal HIOMT levels[12]. However, it has been shown for pineal tryptamines that the diurnal light rhythm is controlled differently in immature and adult rats[13].

Our results add further support to the postulation that the pineal gland may be integrated in the regulation system of adaptation to extreme temperature changes – although the mechanism by which heat alters pineal function is unknown.

*Summary.* Exposure of adult male rats to continuously elevated temperature of 32–34 °C caused a significant decrease of HIOMT activity involved in the specific metabolic process of production of melatonin, considered an active pineal hormone. The effect was already evident after 24 h exposure and increased further during the next 48 h. The results obtained substantiate previous data that the pineal gland may be involved in the system regulating adaptation to extreme temperature changes.

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## PRO LABORATORIO

## Design of a Temperature Controlled Microchamber for Electrophysiological Experiments in vitro

For electrophysiological experiments in vitro, it is essential to control as many environmental parameters as possible. The requirements for each kind of experiments vary[1,2], but most often cultures are transferred to a microchamber which has to be temperature-controlled, have openings for insertion of microelectrodes and be partly made out of transparent material to allow visualization of cells under a microscope. In addition, for our studies of the pharmacological properties of cultured nerve cells, we also required the chamber to be small, the system variably closed or open, and that it be perfused at a constant rate.

This paper describes the design of a microchamber which is easy to construct and to adapt.

*Materials and methods*[3]. The frame of the chamber (Figure 1) is made of anticorodal (Al–Mg–Si-alloy) to obtain adequate heat transfer to the surrounding bathing solution. It is insulated by anodical oxydation to enable the researcher to choose any given electrical potential as a reference point. The chamber has a volume of approximately 1.5 ml ($13 \times 25 \times 5$ mm). These dimensions were found suitable for our needs, since our explants cover an area of less than $6 \times 12$ mm. The bottom of the chamber consists of a glass coverslip glued (by means of Elastosil) to the metal frame, whereas the top is either open or partly closed by another coverslip laid on top of the metal frame and held there by surface tension. Insulated Philips thermocoax NcAc10 (12.5 $\Omega$/m) served as heating wire and was inserted in a single winding in the metal frame around the chamber and reservoir. To ensure good thermal contact and mechanical stability, the space around the thermocoax wire was filled with heat conduction epoxy (E-solder 3025).

The actual temperature of the bathing fluid is constantly recorded by means of a thermistor inserted into one corner of the chamber. A commercially available bridge circuit (Alfos) is used as a control unit. The voltage produced when the bridge becomes unbalanced is amplified and applied to the heating wire. To prevent interference of a/c current with the electrophysiological

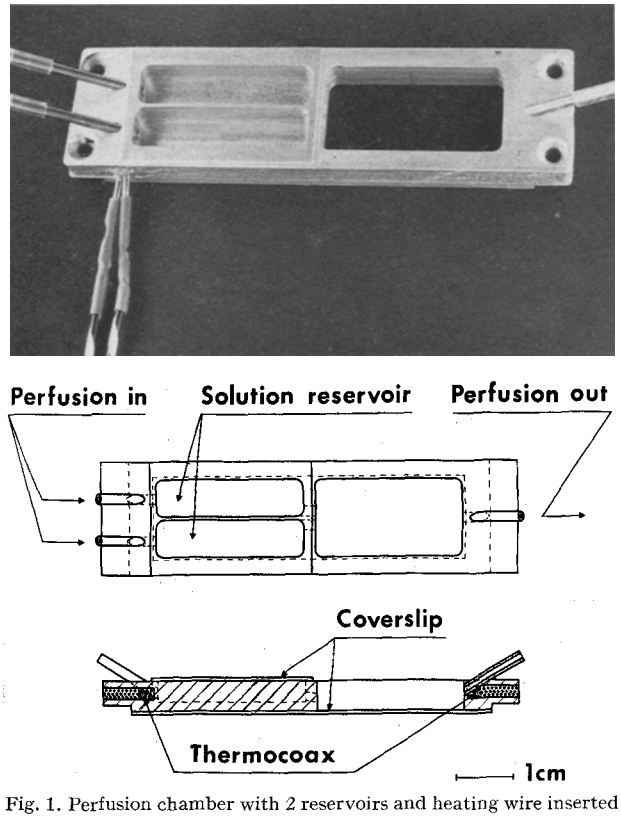


Fig. 1. Perfusion chamber with 2 reservoirs and heating wire inserted into the metal frame.

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