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COMBINATION OF CALORIMETRY AND ENDOSCOPY FOR MONITORING LOCOMOTOR ACTIVITIES OF SMALL ANIMALS

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ABSTRACT

A combination of microcalorimetry and endoscopy is presented and its application to metabolic investigations of small animals is discussed. The endoscopic lighting introduces additional heat production rates of a few milliwatts that are constant under optimum conditions so that they result in base-line shifts. The set-up is tested with snails of low heat production rates. Some further applications after including an image intensifier tube of second or third generation are mentioned.

INTRODUCTION

In recent years calorimetry has gained increasing interest and influence in the biochemical and biological sciences [1,2] but, due to the integrative approach of calorimetry and the lack of specificity of the heat signal, additional equipment is required to differentiate the various heat producing reactions. Among several experimental set-ups optic devices are the most attractive ones. Thus, light guides were used to irradiate microbial cultures with UV light and to measure optical density during growth [3] or to determine substrate concentrations in oscillating reactions [4].

With whole animals the complexity of metabolism increases, so that constant power–time curves (p–t curves) appear or periods of constant heat flow alternate with phases of high activity [5,6]. The question arose whether these temporal structures are due to changes in metabolism or are simply expressions of locomotor activities. Some experiments using electrical stimulation of lizards showed similar shapes in the p–t curves of stimulated animals [7]; and an acoustical observation of honey bees in a calorimeter by means of an incorporated microphone showed simultaneous increases in the

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acoustic level and the rate of heat production [8]. In the course of investigations of the p–t curves of snails [9] we wanted to gain more information about movements of the animals in the calorimetric vessel. Thus it was decided to incorporate a rigid endoscope into the supporting device of the calorimetric vessel and to analyze the conditions for a short-time visual inspection or a long-term monitoring by means of a video camera/recorder system. In this study we report such a combination of Calvet microcalorimetry and endoscopy and the advantages and limitations of this new method.

METHODS AND MATERIALS

The experiments were performed with a Calvet microcalorimeter (Setaram, Lyon, France) with 100-ml vessels and a sensitivity of approx. 60 mV W^{-1}. The time constant of the instrument together with the endoscopic device amounted to 5.0 min. The calorimetric signal was fed to a potentiometric recorder (type BD5, Kipp & Zonen, Delft, The Netherlands).

The endoscope (Boroskop, Storz, Tuttlingen, F.R.G.) with a length of 570 mm, an outer diameter of 6.5 mm, an aperture of 67° and a straight view of 0° was incorporated centrally, parallel to an eccentric supporting rod of the calorimetric vessel. Its end with the Hopkins optic protruded 30 mm into the vessel of a total height of 100 mm. With the large aperture and a depth of focus from 12 mm to infinity, almost the whole volume of the vessel could be inspected. The device was illuminated using a light guide and the endoscope by a special cold light projector of 150 W (Storz, Tuttlingen, F.R.G.) with three levels of intensity.

As these levels are far too high for calorimetric experiments, rendering unnecessarily strong illumination and thus heat flows to the vessel, a support for filters was placed between the light guide and the endoscope. Using neutral filters (type NG9, Schott, Mainz, F.R.G.) the illumination could be reduced to a minimum level for visual observation or video monitoring. Neutral filters are necessary as colored glasses would change the spectral distribution of the light and might provoke responses of the animals to such changes.

A black-and-white video camera (Panasonic, Model WV-1800C, Matsushita, Tokyo, Japan) with a minimum required illumination of 2 lux and a recommended illumination of 20 lux was directly adapted to the eyepiece of the endoscope. The signal was monitored by a video recorder (HR-3660EG, JVC, Tokyo, Japan). Film sequences of slowly moving animals could be inspected by a quick-motion technique (ratio 1 : 2). Because of the high sensitivity of the dark-adapted eye the illumination level for video recording had to be higher by a factor of seven than for direct observation.

Photographs were taken with a directly connected camera (type OM2, Olympus, Hamburg, F.R.G.) at open diaphragm and a film of high sensitiv-

ity (400 ASA, Ilford HP5). Typical settings were level I, absorption 0.85, 20 sec exposure (corresponding to approx. 1 lux).

RESULTS AND DISCUSSION

Figure 1 shows the situation in the calorimetric vessel viewed through the endoscope. The snail with an artificial white marking line on the shell is clearly visible. The vessel bears six vertical lines (one a triplet) at spacings of 60°, and two vertical rings 1.5 and 3.0 cm above the bottom. They may serve as markers for monitoring the movement of the animal within the chamber. The rings enable one to estimate upward creeping of the snail, that happens from time to time and leads to pronounced changes in the power–time curves (see Fig. 4). Moreover, one realizes the excellent depth of focus due to the Hopkins optic which covers the whole field of vision under these experimental conditions.

The heat production rates at the different levels of illumination were determined with empty calorimetric vessels. The results are given in Table 1. With level I and a neutral filter of 0.995 absorption all details in the vessel are seen even in daylight rooms, while with level I and 0.999 absorption the eye has to adapt for some time in a moderately darkened environment. Clear

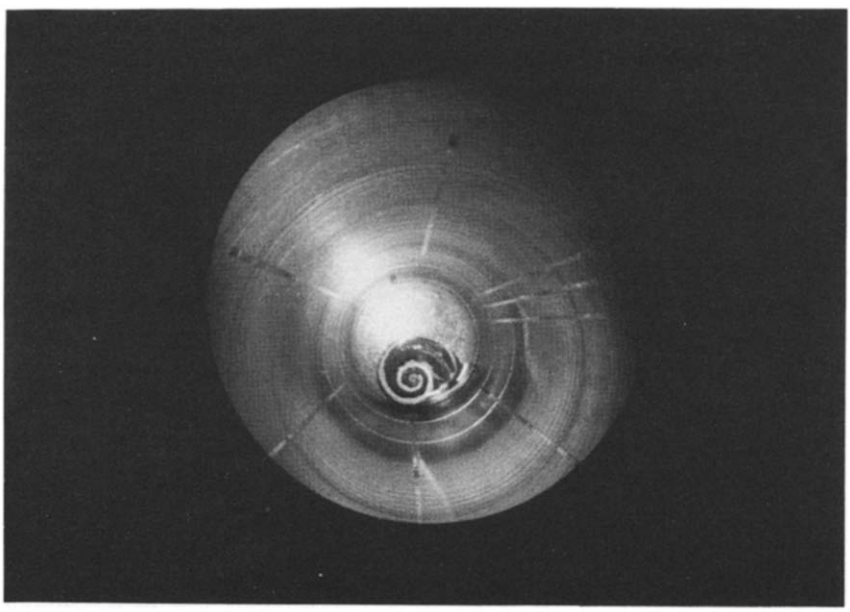

Fig. 1. Endoscopic view of the snail at the bottom of the calorimetric vessel. The snail is marked with a white line. For better orientation six vertical lines and two horizontal rings as described in the text are scratched on the walls.

TABLE 1

Heat production rates for illumination without and with neutral filters of differing grades of absorption

Parameters	Heat production rate (mW)
Without filter	
Level I	55.2
Level II	73.8
Level III	94.6
With filter (level I)	
0.995 absorption	2.22
0.999 absorption	1.02
0.9995 absorption	0.69
0.9999 absorption	0.37

observations are only possible at 0.999 absorption and level II, i.e. at illuminations rendering a heat production of 1.35 mW.

The video camera needs at least illuminations of level I and 0.850 absorption, i.e. additional heat production rates of 10 mW. Modern cameras are sensitive down to illumination levels of 1 lux but need a recommended lighting of 10 to 20 lux for clear pictures. (For comparison: in working rooms with very low demands for the viewing task, illumination levels of 30 lux are prescribed by the German norm DIN 5035.) A further significant step down to extremely low illumination levels can be achieved using modern image intensifiers of the second or third generation which are used for military observation or hunting purposes and which are sometimes sold as "moonlight cameras". These image intensifiers render amplifications up to approximately 50000 and thus enable observations at 10^{-5} lux. Such illumination levels correspond to star light on a clear winter night.

Absolute figures of heat production are meaningless until compared with the biological signal which is registered. Table 2 shows the minimum illumination levels for visual and video observations together with heat production rates for some small animals. Among the animals cited here snails are critical because of their very low basal metabolism.

The disturbances introduced by the endoscope become less important when one looks to the temporal fluctuations of the illumination signal (Fig. 2). Without additional stabilization of the light source the rate of heat production changes less than 3.5% during 2 h, while after stabilization the fluctuations are smaller than 0.45% or 0.010 mW at level I and 0.995 absorption. Thus illumination produces a constant shift of the base line which is even not detectable in experiments with bees, when the experiments are run under reduced sensitivities because of strong locomotor activities. Some disturbances are introduced by slight changes in room temperature due to the action of room climatization. Without climatization the "noise"

TABLE 2

Mean heat production by illumination and by some small animals which have been observed endoscopically

		Heat production rate (mW)
Visual observation		1.35
Video monitoring		10.1
Snail (without shell)	500 mg ww	0.37 [9]
Honey bee	200 mg	15.8 [8]
Cricket	300 mg	1.67 [10]
Lizard	4000 mg	8.04 [7]

(ww = wet weight).

drops to less than 0.006 mW during 4 h, which is less than 2% of the main snail signal (Fig. 2). Additional thermal shielding avoids these problems at high sensitivities.

Figures 3 and 4 show some preliminary results for the correlation of heat production rate P and locomotor activity of snails of the species *Biomphalaria glabrata*. Some characteristic points are indicated by arrows and + (activity) and − (rest) signs and a differently turned spiral indicating the instantaneous position of the shell (Fig. 3). In most cases the snail lies vertically at the bottom in a layer of 5 ml water (0.7 cm high). In some cases, indicated by a solidus (/), the shell stands perpendicular to that orientation. The small ripples in the p–t curves are clearly connected with the movement of the snail, whilst the long-lasting features at the beginning and the end of the curve must be due to other, supposedly metabolic processes.

Figure 4 exhibits a situation with greater long-period fluctuations in the rate of heat production. At the beginning the snail is in an upright position,

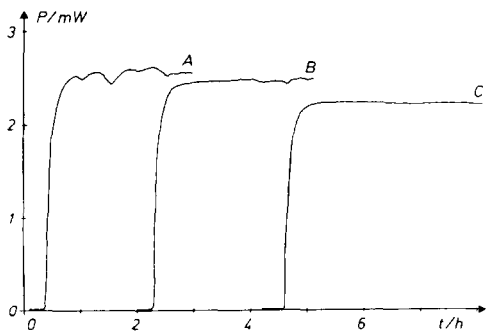

Fig. 2. Heat production by endoscopic illumination at level I and an absorption of 0.995 by a neutral filter. A, with climatization and without stabilization (noise ±3.5%); B, without climatization and without stabilization (noise ±1.4%); C, without climatization and with stabilization (noise < ±0.3%).

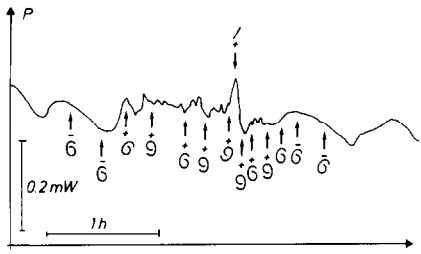

Fig. 3. Power–time curve of a snail under endoscopic observation. +, movement with horizontal shell; −, no detectable movement; /, shell in a vertical position under this orientation; 6 , orientation of the snail in the vessel.

then it drops to its back (notice the other sense of spirality!), moves around and stands up again, this time coming back to the correct vertical position. A phase of strong activity follows during which the snail leaves the water layer (∠) and creeps up the wall to the top of the vessel. Later it returns to the water and remains in an upright position without any detectable movement while changes in the rate of heat production are recorded. These features with a relatively quick increase and a rather slow and nearly-linear decrease are of special interest [9–11]. It becomes evident from these observations that the changes must be connected with some periodic events of metabolism as discussed in refs. 9 and 10, and not just due to locomotor activities. Further investigations by combined endoscopy and image intensification are planned to address such questions.

Although the levels of heat production due to illumination during visual or video observations are low enough for most calorimetric measurements, these illuminations may be still too strong for the physiological responses of the animals. Most organisms are phototactic, i.e. they turn to the light and show varying activity responses to changing light conditions. Moreover, most calorimetric experiments performed to date have been run in closed batch vessels in complete darkness, so that features in their p–t curves

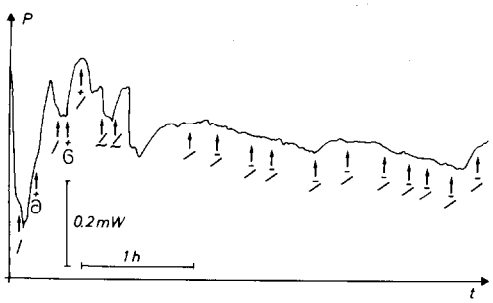

Fig. 4. Power–time curve of a snail. See Fig. 3 for the explanation of the symbols, and the text for the significance of the features of the curve.

remain unexplained by this method. In such cases it would be interesting and necessary to couple the video camera with an image intensifier as mentioned above and decrease the illumination level at least by a factor of 10^3, and under extreme conditions by up to 10^5.

With such a set-up a new field of physiological calorimetry would be opened: investigation of energy metabolism under increasing strength of illumination (from night to daylight conditions) becomes possible. Moreover, features in the p–t curves can then be attributed to special types of locomotor activity. It was stated for snails [12] that 80% of energy turnover is due to movement. The results from Figs. 3 and 4 seem to contradict such findings. The typical slow movement of a snail in the calorimetric vessel introduces only small ripples in the p–t curves so that the strong fluctuations of heat production with periodicities of 2–3 h [9,10] must be due to other activities (metabolic ones or locomotion within the shell), which have so far not been recorded.

Due to the thermal inertia of the calorimeter, the features in the p–t curve are damped and are not as pronounced for quick changes as the original signal. This problem is often overcome in calorimetry by "desmearing" techniques [13,14]. With visual observation one possesses an instantaneous technique which allows for a subtle investigation of the actual state of movement.

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