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OPTIMIZATION OF CALORIMETRIC SYSTEMS: CONTINUOUS CONTROL OF BASELINE STABILITY BY MONITORING THERMOSTAT TEMPERATURES

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ABSTRACT

Despite the employment of modern twin calorimeters, the evaluation of baseline stability remained one of the outstanding methodological problems in biological microcalorimeters operated near the limit of detection. Baseline instabilities are mainly caused by thermal disturbances from environmental and thermostat fluctuations. Therefore continuously monitoring the temperatures in the thermostat and heat sink provides a good indication of the reliability of calorimetric measurements. In addition, numerical methods are available to correct the heat flow curves from temperature disturbances. Such temperature correction procedures can improve the accuracy of various types of microcalorimeters.

INTRODUCTION

Many refinements of microcalorimeters broadened the range of application of this method beyond the limits previously set by insufficient sensitivity. The main improvement of heat flow calorimeters with thermopiles as the heat detector was achieved by the automatic compensation of external disturbances: In twin systems these are largely canceled by obtaining a difference signal between the experimental heat detector and the symmetrically arranged static reference detector (Calvet and Prat, 1963; Wadsö, 1970). Accordingly, the baseline stability and hence the long term detection limit of twin calorimeters are set by symmetry characteristics and by the constancy of the thermostat. Both can be improved within limits only.

High long term stability of heat flow measurements, however, is required in many applications and is especially important in biological microcalorimetry with flow through systems (Gnaiger, 1979). Extremely small heat effects have to be detected in the measurement of maintenance metabolism of microorganisms (Forrest and Walker, 1963) and in the study of heterotrophic activity in the natural aquatic environment. Direct calorimetry represents the only unspecific method for the quantification of aerobic and anoxic metabolism (Gnaiger, 1979, 1980a; Pamatmat, 1979) and the reduced anoxic heat effects (Gnaiger, 1980b) entail the necessity of employing a high-precision calorimeter. Moreover, the recent development of an open-flow system for the simultaneous measurement of oxygen consumption and heat dissipation of aquatic organisms (Gnaiger, in press) provides the method required to solve a fundamental problem in ecological energetics: How far is indirect calorimetry (= the measurement of oxygen consumption) sufficient and reliable for estimating the heat flux in ecological systems?

The evaluation of the accuracy of long term heat flow measurements is a prerequisite for the quantitative comparison of the direct and indirect calorimetric results. It proved necessary to obtain a continuous indication of system functions. For the microrespirometer this is achieved by employing a new twin-flow principle (Gnaiger, in press). A generally applicable method is advanced in this article to monitor the thermostat and heat sink temperature as an indication of the calorimeter baseline stability. We also present procedures not only to control but also to improve the stability of a twin-microcalorimeter by mathematically correcting the power-time curves for external disturbances of the baseline.

THE LKB 2107 FLOW MICROCALORIMETER AND TEMPERATURE REGISTRATION

The LKB 2107 is a Wadsö type isoperibolic twin-microcalorimeter equipped with a flow sorption vessel. The operation principle is shown in Fig. 1. The calorimeter is placed in an airbath thermostat regulated at ± 0.01°C, and consists of two symmetrically arranged detector systems (D, R) and a thermally isolated 12 kg aluminium heat sink (H). The detector (D) is a small brass block where the 0.5 cm^3 pyrex chamber (A) is inserted. Heat effects are measured as a temperature gradient over the semiconductor thermopiles (T_D, T_R). A detector heat exchanger (E_D) ensures the heat transfer between the flow medium and the detector. The measurement and the reference detector are wired differentially to cancel out symmetrical disturbances. The sensitivity is about 0.05 $\mu V.\mu W^{-1}$. The resulting output voltage is amplified by a Keithley 150B amplifier.

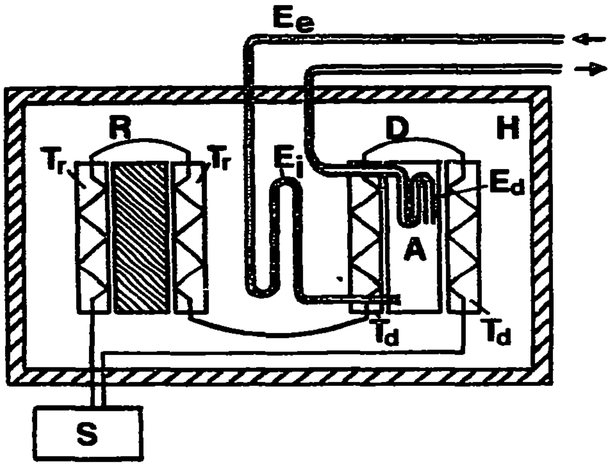

Fig. 1: Operation principle of the flow-microcalorimeter LKB 2107

A	Pyrex vessel 0.5 cm^3	H	Heat sink
D	Detector	R	Reference detector
E_D	Detector heat exchanger	T_D	Detector thermopile
E_E	External heat exchanger	T_R	Reference thermopile
E_I	Internal heat exchanger	S	Amplifier

The flow medium (water) was sucked through the system at a constant flow rate of 3 to 15 $cm^3 . h^{-1}$ after equilibration at $\pm$ 0.1°C of operating temperature (which was between 8 and 20°C). Further equilibration was performed by heat exchangers located in the thermostat (E_E) and the heat sink (E_I).

Absolute temperature was measured to $\pm$ 0.1°C using YSI Thermilinear thermistors. Relative changes were obtained at a precision of $\pm$ 0.002°C.

SOURCES OF BASELINE DISTURBANCES

Baseline fluctuations are mainly caused by various thermal disturbances. Short fluctuations of thermostat temperature are filtered by the thermal insulation of the heat sink, but stability is required to avoid erroneous heat flux across the detectors. This demand was not sufficiently accomplished.

The twin-compensation method requires both detectors to be symmetric in their apparatus function (sensitivity, time constant) and thermal capacity. The thermal capacity was disturbed because the reference detector did not contain animals and flow medium.

In addition asymmetric disturbances of the detectors cannot be compensated by a twin-compensation method. Errors of this kind may be caused 1) by poor equilibration of the flow medium, wherefore the system behaviour will depend on the medium flow rate; and 2) by thermal leakage from the air-bath to the detector through the tubings. This problem seems rather serious because the originally employed teflon tubing had to be replaced by gold capillaries to avoid gas diffusion for simultaneous respirometry.

CONTINUOUS CONTROL OF BASELINE STABILITY

Room temperature was highly correlated with baseline fluctuations (Fig. 2). The corresponding time derivatives were used to estimate the cross-correlation function (Fig. 3). The calorimeter baseline followed the temperature with a delay of approximately 1h. Therefore the room temperature was stabilised to $\pm$ 1°C.

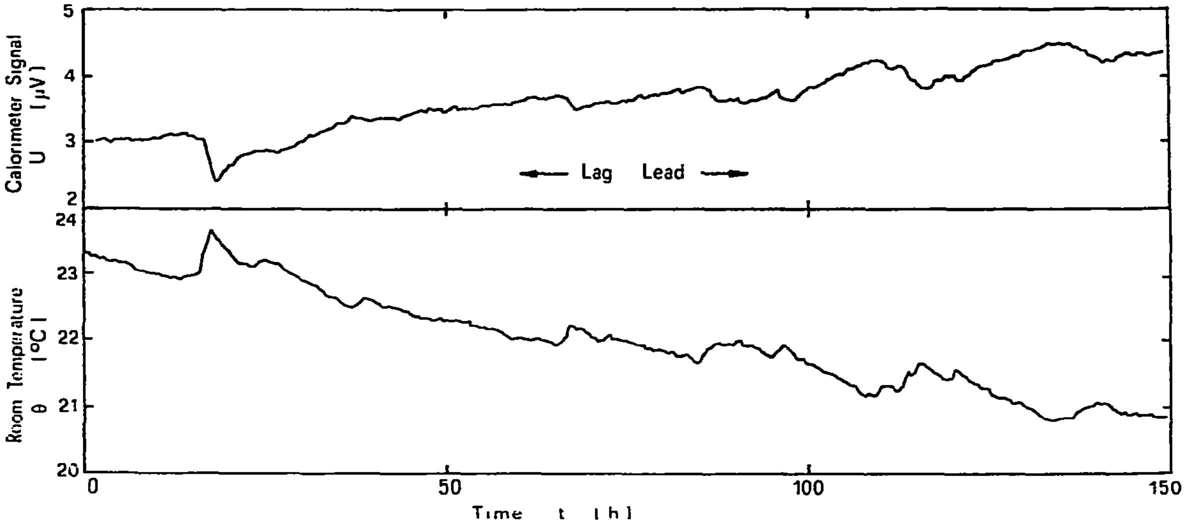

Fig. 2: Simultaneous trace of environmental temperature and calorimeter baseline. The obvious correlation clearly necessitates stabilisation of the room temperature.

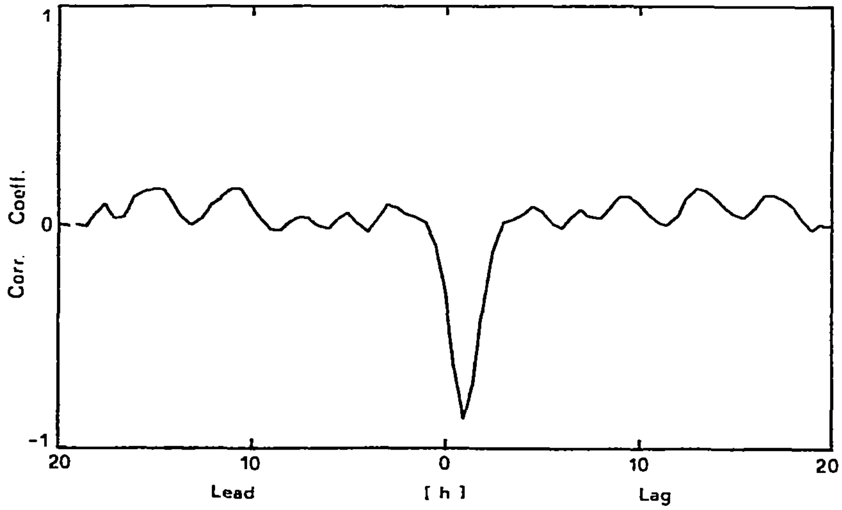

Fig. 3: Cross correlation of room temperature and calorimeter signal as a function of calorimeter lead and lag estimated from the differentials of the records of Fig. 2. The sharp peak (R = 0.876) illustrates the 1h delayed response of the calorimeter.

In a further step the air-bath thermostat temperature was continuously recorded. The short term stability of about $\pm$ 1.10^{-3}°C could not be maintained in long term experiments where drifts and fluctuations of $\pm$ 5.10^{-3}°C occured. These were related to the baseline disturbances (Fig. 5).

A further improvement of baseline behaviour was achieved by symmetric layout of the gold tubing into the heat sink. Fig. 4 shows the baseline drift of 0.1 µV over a period of 16 h and its correlation with thermostat temperature drift of 5.10^{-3}°C.

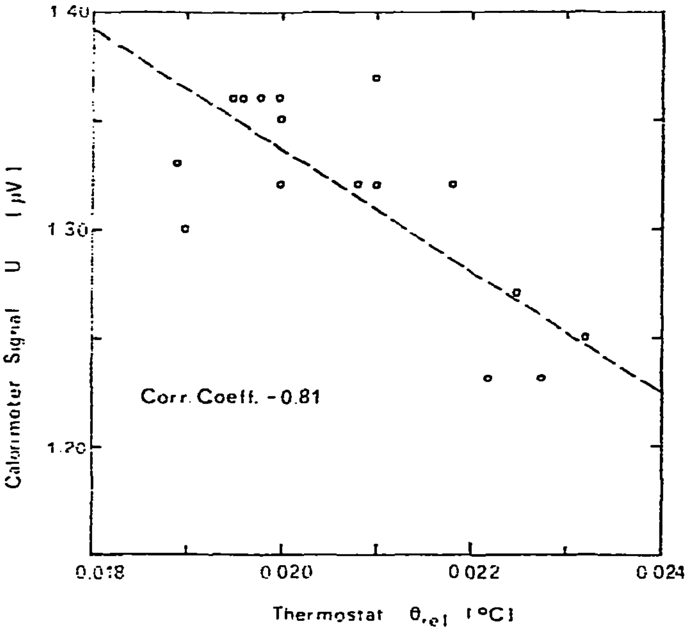

Fig. 4: Correlation of spontaneous thermostat temperature drift (0.005°C) and baseline drift (0.1 µV ≙ 1.8 µW) over a period of 16 h not considering time delays.

BASELINE CORRECTION

The close correlation of baseline instabilities and changes in thermostat temperature entails the possibility of correcting the power-time curves for external disturbances. An experiment with 4 eggs of the salmonid fish Salvelinus alpinus was conducted with an asymmetric arrangement of the gold capillary tubes (see above). Under these conditions, changes of the calorimeter signal in a constant environment apparently surmounted the metabolic heat dissipation under reduced experimental p_{O_2}. This variability originated from irregularities of the thermostat (Fig. 5). A simple plot of the calorimeter output vs. thermostat temperature permits the discrimination between systematic errors of measurement and biological heat effects (Fig. 6). Despite the unfavorable conditions in these experiments, the difference between the plots for the three environmental oxygen situations corresponded well with more reliable results (Gnaiger, 1979).

The system's short term reaction to thermostat disturbances was determined by observing its response to an artificial temperature pulse (Fig. 7). A well-behaved transfer function was obtained which remained constant within any experiment under constant conditions. It may be used to correct mathematically the heat flow measurements either by using Fourier transform methods or the recursion method proposed for "de-smearing" by Höhne (1978).

Long term system behavior was investigated employing a temperature step of $-1^{\circ}C$ in the thermostat (Fig. 8A). The heat sink equilibrates rather slowly ($\tau \approx 12$ h) because of its thermal capacity and insulation against the air-bath. The relation of heat sink temperature change and calorimeter signal (Fig. 8B) seems quite useful to correct baseline drifts caused by thermostat instability or slow initial equilibration.

A different system has been used by Pamatmat (1979) to correct for baseline drift in a double-twin calorimeter. Here a second twin chamber within the same heat sink is monitored to detect instabilities of the experimental twin chamber. This method rests on the assumption that the poor symmetry of one twin system is identically reproduced in the second one, i.e. that both react in a similar manner to external influences. Experimental tests of this assumption are required. Compared to the double-twin system the temperature correction method proposed in this work will yield a similar improvement of baseline accuracy at a much lower expense.

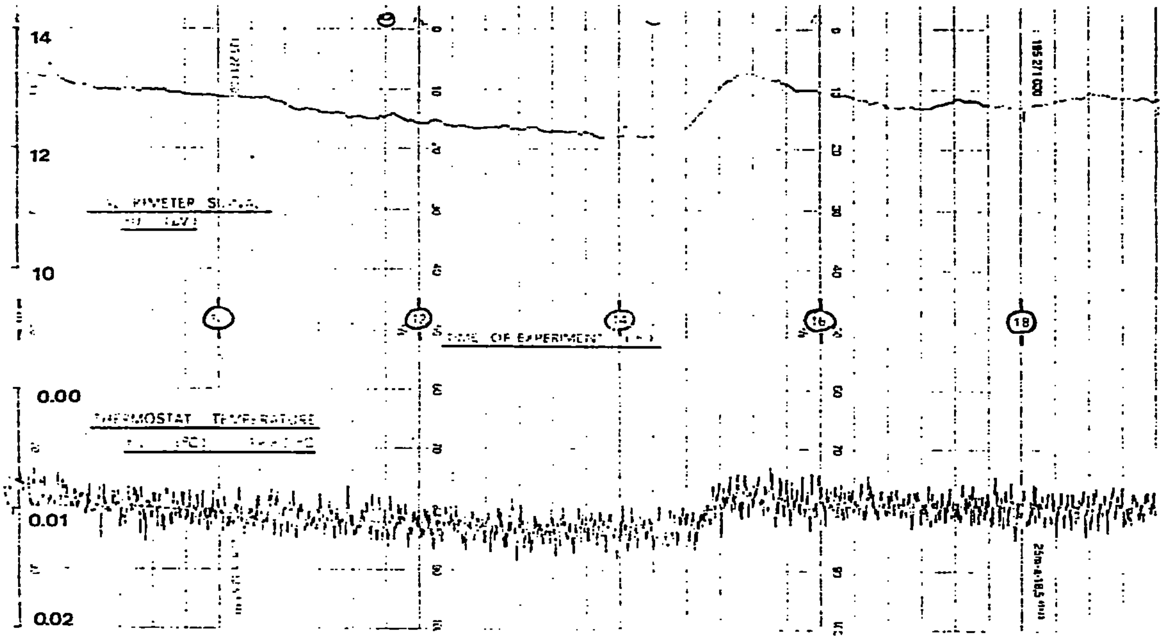

Fig. 5: Calorimeter and thermostat temperature records in an experiment with Salvelinus alpinus (4 embryos, 8.0°C). The 10-fold larger influence of the thermostat compared to Fig. 4 is explained by the poor symmetry of the gold capillary tubing in this experiment.

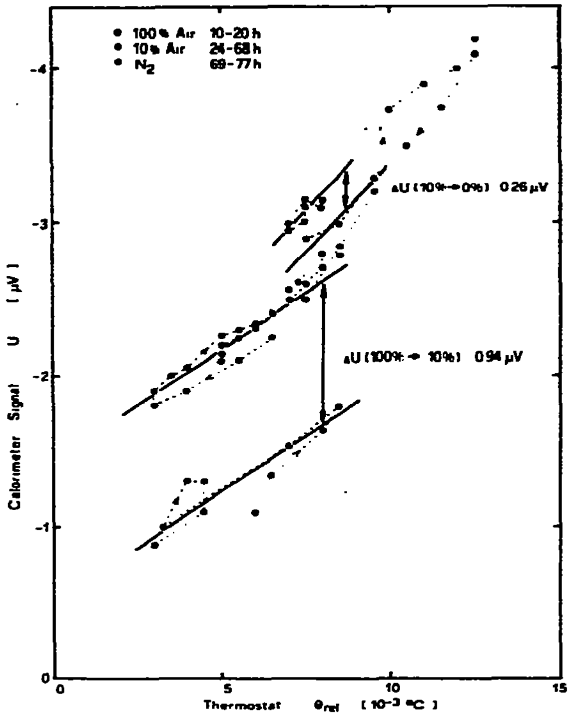

Fig. 6: The plot of calorimeter output versus thermostat temperature (data from Fig. 5) permits the discrimination of heat dissipation at 100 and 10% air saturation and of the levels at 100% and pure nitrogen.

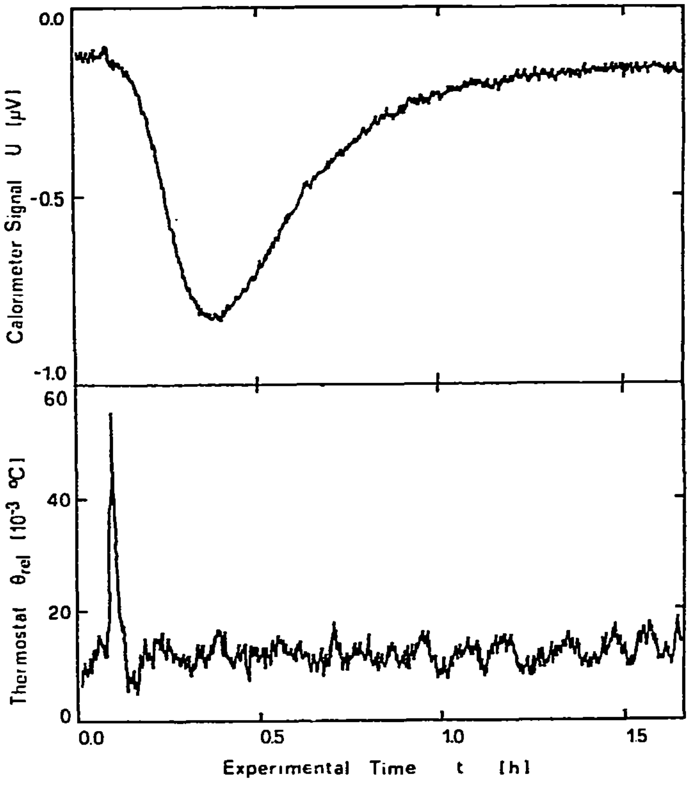

Fig. 7: Calorimeter response to an artificially induced short term temperature pulse in the thermostat. This experimentally derived transfer function can be used in baseline correction to account for the transient behavior of the system (time series analysis methods).

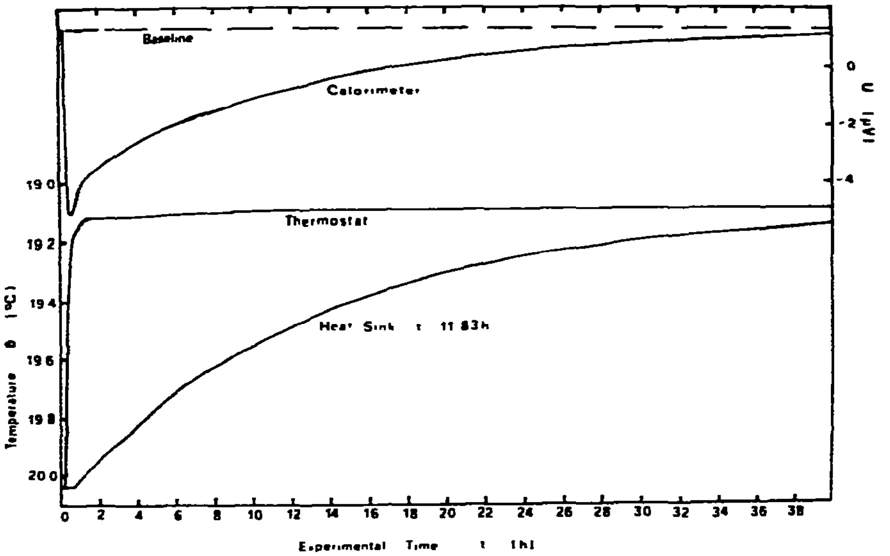

Fig. 8A: Response of calorimeter output and heat sink temperature to a $-1^{\circ}C$ step of thermostat temperature. The calorimeter returns to its original baseline as the heat sink equilibrates ($\tau \simeq 12$ h)

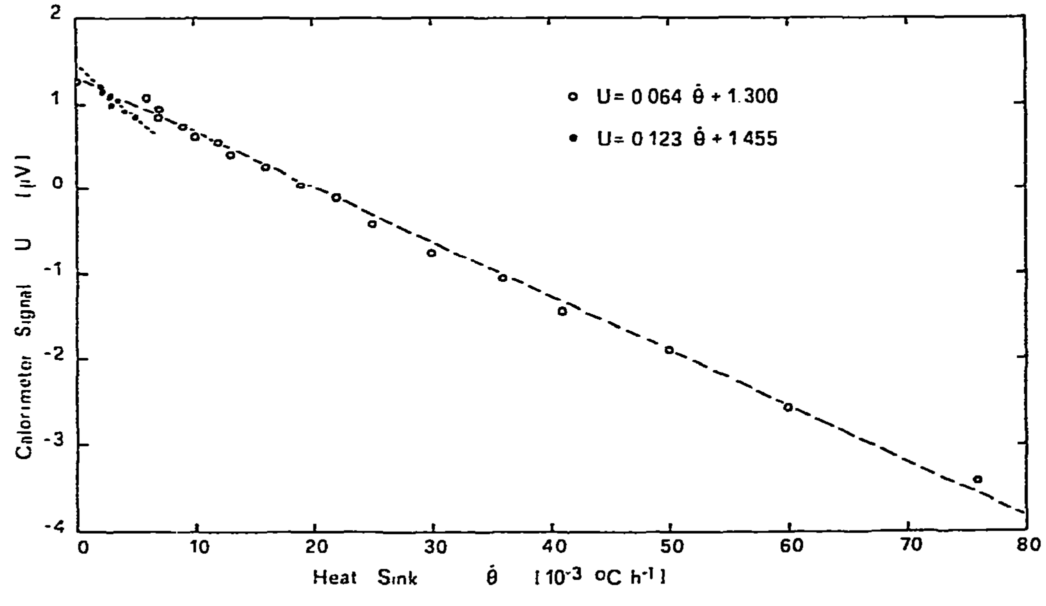

Fig. 8B: Relation of heat sink temperature change and calorimeter signal. o Data from Fig. 8A. The regression intercept is identical with the original baseline value. • Data from another experiment with small temperature changes. This system behavior may be used to correct a major part of baseline drift.

SUMMARY

1.) The continuous monitoring of temperature disturbances revealed the importance of increasing thermostat stability for obtaining a sufficiently constant baseline as required in many biological applications.

2.) Improvement of the symmetry properties of the twin-system presents an additional means of ensuring a stable baseline. This is especially important in flow calorimeters employing metal capillary tubing. The efficiency of the twin-compensation was tested by correlations of thermal fluctuations and baseline instabilities.

3.) Optimization of these physical characteristics is restricted. As an alternative we propose to use continuous temperature records and numerical methods of time-series analysis to correct for residual system instabilities. In fact this software approach is attractive for reasons of economy, since the same methods can be applied in time-lag corrections of the experimental signal and for interpreting the biological experiments.

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