Thermochimica Acta, 160 (1990) 173–192
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

DETERMINATION OF PHASE CHANGES AND METABOLIC RATES IN PLANT TISSUES AS A FUNCTION OF TEMPERATURE BY HEAT CONDUCTION DSC

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(Received 22 August 1989)

ABSTRACT

Heat conduction differential scanning calorimetry methods have been developed for the continuous measurement of metabolic heat rates of plant tissues as a function of temperature. Thermally induced transitions and metabolic heat rate can be determined simultaneously. As examples of the utility of this method, data are given on metabolic heat rates measured as a function of temperature for barley root tips and cultured tomato cells.

INTRODUCTION

Calorimetry has long been recognized in biological applications as a useful method for determining metabolic rates. The metabolic rate of an organism can be used as an indicator of the effects of various kinds of stresses on the organism, the rate of growth of the organism, and of the biochemical pathways being used by the organism [1]. The effect of temperature on the metabolic rate is often of interest in such studies. Several years ago, Loike et al. [2] showed that temperature scanning calorimetry could be used to obtain data rapidly on the metabolic rate of mammalian cell culture as a function of temperature. These workers also pointed out an ambiguity present in their studies, i.e. the data provided no general basis for deciding whether a change in the calorimeter output was due to variation in the metabolic heat rate or in the apparent specific heat of the cells. With the limitations of existing calorimeters, it was not possible to overcome the ambiguity. However, temperature scanning calorimeters with a wider range

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0040-6031/90/$03.50

of scan rates and the ability to scan down as well as up are now available and can be used to eliminate ambiguity.

A significant question in the biology of green plants is how tissues are damaged by high and low (but not freezing) temperatures. When chilled, sensitive plants, e.g. tomato, cease to grow and may eventually die if exposed to temperatures below 10–12 °C. Plants are also sensitive to temperatures above 35–40 °C where they undergo heat shock responses. The current most popular hypothesis used to explain the existence of abrupt changes in metabolism with temperature is a lipid phase change in the cell membranes [3]. However, the evidence for this hypothesis is circumstantial and thus is open to serious question [4]. In a previous paper [5], we have shown that isothermal calorimetric measurements of dark metabolic heat rates accurately reflect plant responses to high and low temperatures. This study examines whether similar data on metabolic heat rates are obtainable from temperature scanning experiments, and whether thermal signals resulting from physical changes in components of the tissues during the temperature scan can be detected simultaneously. Such experiments could conclusively show whether the physical events are coincident with, and thus the probable cause of, the metabolic changes. This paper describes methodology for measuring metabolic heat as a continuous function of temperature, develops methods for estimation of the errors involved in, and thus the optimization of, the application of scanning calorimetry to measurement of metabolic rates in living systems, and establishes conditions under which the thermal effects of physical changes can be distinguished from metabolic effects.

DATA ANALYSIS

A schematic diagram of a differential temperature-scanning heat conduction calorimeter (DSC) is shown in Fig. 1. Equation (1) describes the output signal from the calorimeter

$$P = k_S \, \Delta T_S - k_R \, \Delta T_R \tag{1}$$

where P is the measured thermal power, k is the thermal conductivity between an ampule and the block, ΔT is the temperature difference between an ampule and the block, subscript S refers to the sample side, and subscript R refers to the reference side of the calorimeter. During a temperature scanning experiment, the temperature of the block is changed with time as given in eqn. (2):

$$T = T_0 + B(t)$$

and

$$(\mathrm{d}T/\mathrm{d}t) = (\mathrm{d}B(t)/\mathrm{d}t) = b \tag{2}$$

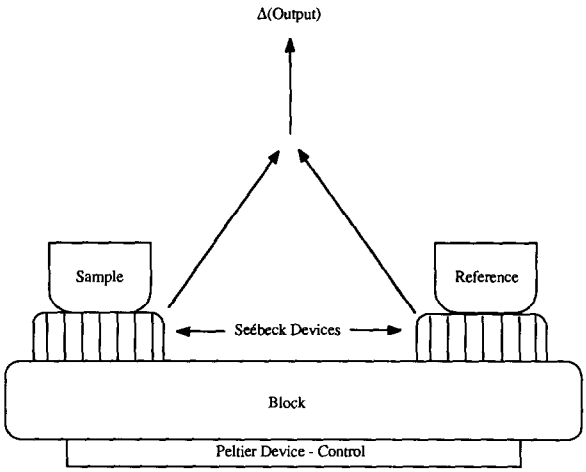

Fig. 1. Schematic diagram of a differential temperature-scanning heat conduction calorimeter.

where T is the block temperature, T_0 is the initial block temperature, $B(t)$ is a function of time, b is the rate of change of the block temperature with time, and t is time. It should be noted that b is not necessarily a constant and may be expressed as a function of time or temperature. Under conditions where the Tian correction is negligible [6], or if the Tian correction has been applied to P, eqn. (3) describes the heat flow to or from the ampules:

$$P = b(C_A + C_S) + q_S - b(C_R + C_C) \tag{3}$$

where C_A is the heat capacity of the sample ampule, C_S is the heat capacity of the sample in the ampule, q_S is the rate of heat produced or absorbed by any reactions taking place in the sample, C_R is the heat capacity of the empty reference ampule, and C_C is the heat capacity of anything added to the reference ampule in order to reduce the heat capacity difference between sample and reference ampules and thereby improve the instrument performance. The purpose of this study is the determination of q_S as a function of temperature.

A determination of q_S requires a minimum of two sets of temperature scans: a baseline scan with both ampules empty and a scan with sample present in one ampule. The measured power in the baseline scan is described by eqn. (4):

$$P_B = b_B(C_A - C_R) \tag{4}$$

The scan with sample present is described by eqn. (5):

$$P_S = b_S(C_A + C_S) + q_S - b_S(C_R + C_C) \tag{5}$$

Equations (4) and (5) are easily derived from eqn. (3). Since the scan rates, b_B and b_S, are not necessarily equal, eqns. (4) and (5) must be rearranged to

give the heat capacity, i.e. P/b, before the baseline data can be subtracted from the sample scan data as shown in eqn. (6):

$$(P_S/b_S) - (P_B/b_B) = P_C = (C_A + C_S) + q_S/b_S - (C_R + C_C) - (C_A - C_R)$$
$$= (q_S/b_S) + C_S - C_C \quad (6)$$

The quantities P_S, b_S, P_B, and b_B are available from the data measured during the temperature scans, i.e. temperature, time, and heat rate. Values for C_S and C_C must be obtained from other experiments. C_C can be chosen to be zero by adding nothing to the reference ampule or may be calculated from literature data if a known amount of a material with a known heat capacity is added to the ampule.

The precise determination of the effective value of C_S to be used in a given experiment is more troublesome. Previous unpublished work by us and others has shown that the absolute determination by DSC of both C_S and the temperature dependence of C_S is inaccurate. Plots of C_S values determined by DSC vs. temperature show that the curves have the correct shape, but the absolute positions of the ends of the curves can be in error by several per cent. Thus, if the endpoints can be accurately established by means of an independent method, the entire curve of C_S vs. T will be accurately established. We have therefore examined all of the possible methods for determination of C_S. In some situations, calculation of C_S from literature data may be possible, but this is unlikely for samples of plant materials as studied here. C_S may be determined in a separate experiment on the same sample where the scan rate, b_S, is chosen to be large enough so that q_S/b_S is negligible compared with C_S (see eqn. (3)); it may be calculated from eqn. (6) and a separate, isothermal measurement of q_S; or it may be determined from the intercept of a plot of P_C against $1/b_S$ data for several scans done at different rates. All these methods may be useful under various circumstances depending upon the exact nature of the samples being studied and the desired use of the results, but only the separate, isothermal measurement of q_S [5] at both ends of a temperature scan proved to be a practical, effective way of obtaining accurate C_S values at both ends of the temperature scan. Then it must be assumed that C_S is described by a continuous simple function, such as a linear or quadratic function, over the scan temperature range.

After the sample heat rate, q_S, has been obtained it must be further broken down into q_M, the rate of heat generated by metabolism, and q_p, the rate of heat generated or absorbed by any thermally induced phase changes in the sample (eqn. (7)):

$$q_S = q_M + q_p \quad (7)$$

This can generally be accomplished because overall metabolic heat is always exothermic irrespective of scan direction, while phase changes will generally be endothermic on upward scans and, if reversible, exothermic on downward

scans. Thus, values of q_M and q_p will often be separable in data collected by scanning in both directions. If the temperature range scanned extends beyond both ends of the temperature range of the phase change, q_M and q_p are then also separated in time and the problem is simplified. In the absence of any irreversible physical changes, the heat capacity function, i.e. $C_S(T)$, must be the same for an upward scan as for a downward scan. Therefore, no significant information is lost in obtaining a baseline with the assumption that C_S is a continuous simple function. In the case of a first-order phase transition, $C_S(T)$ is actually discontinuous, $\Delta H = T\Delta S \neq 0$, and a thermal signal for the transition will always exist [7,8]. Calculation of ΔH by integration of the peak in a q_S vs. t plot will be somewhat in error, however, because of the assumption of a continuous $C_S(T)$. In the case of a λ (higher order) transition, $\Delta H = T\Delta S \approx 0$, and $C_S(T)$ has a cusp-type discontinuity [7,8]. Because of the assumption of a continuous function for $C_S(T)$, the discontinuity will appear as an endothermic peak on the assumed baseline. Any calculation of ΔH from such a peak would, of course, be totally in error. Still, the λ phase change would result in a thermal signal. Whether the thermal signal is seen or not depends on the size of the signal, calorimeter sensitivity, and optimization of experimental variables.

OPTIMIZATION OF EXPERIMENTAL VARIABLES

Selections of scan rate and sample size are crucial to the success of an experiment. The metabolic heat rate q_M will dominate at slow scan rates while the C_S and q_p terms will be dominant at fast scan rates. Also, practical considerations of sample viability must be taken into account because oxygen or some other metabolite may be exhausted or the metabolism may be altered by build-up of a metabolic product before the experiment is completed, if scan rates are slow. The kinetics of adjustment of metabolism to changes in temperature must also be considered. Thus, conditions must be optimized for each experiment. This requires that a general error analysis be done on the above equations.

Solving eqn. (6) for q_S gives eqn. (8):

$$q_S = b_S(P_C - C_S + C_C) \tag{8}$$

Error analysis of eqn. (8) results in eqn. (9):

$$\begin{aligned}\delta q_S &= \left[b_S^2\left(\delta P_C^2 + \delta C_S^2 + \delta C_C^2\right) + \left(P_C - C_S + C_C\right)^2 \delta b^2\right]^{0.5} \\ &= \left[b_S^2\left(\delta P_C^2 + \delta C_S^2 + \delta C_C^2\right) + \left(q_S/b_S\right)^2 \delta b^2\right]^{0.5}\end{aligned} \tag{9}$$

where δ indicates the standard deviation of the indicated quantity. Error analysis of the leftmost side of eqn. (6) gives eqn. (10) for evaluation of δP_C.

It should be noted that values for P_B/b_B and P_S/b_S are obtained from eqns. (4) and (5), respectively:

$$\delta P_C = \left[(\delta P_S/b_S)^2 + (\delta P_B/b_B)^2 + (P_S/b_S)^2(\delta b_S/b_S)^2 + (P_B/b_B)^2(\delta b_B/b_B)^2\right]^{0.5} \quad (10)$$

The scan rate b is approximately equal to $\Delta T/\Delta t$ so δb is given by

$$\delta b = \left[(\delta\Delta T/\Delta t)^2 + (\Delta T/\Delta t^2)^2(\delta\Delta t)^2\right]^{0.5} \quad (11)$$

The sample heat capacity C_S is given by the product of the heat capacity per gram, C_W, and the mass of the sample, W_S. Therefore, the error is given by

$$\delta C_S = \left[(C_W\delta W_S)^2 + (W_S\delta C_W)^2\right]^{0.5} \quad (12)$$

In studies in our laboratory, a copper slug is usually added to the reference ampule to make sample and reference heat capacities comparable. The heat capacity of the Cu slug, C_C, is calculated as the mass, W_{Cu}, times the heat capacity per gram, C_{Cu}, so the error in C_C is given by

$$\delta C_C = \left[(C_{Cu}\delta W_{Cu})^2 + (W_{Cu}\delta C_{Cu})\right]^{0.5} \quad (13)$$

Table 1 gives the estimated errors and range of typical values for the various quantities in eqns. (9)–(13) for the calorimeter and materials used in this study. Values for b, Δt, W_S and the temperature range to be scanned must be selected for each experiment. Proper choice of these values must be made in order to minimize the error in the determination. Appendix A lists a BASIC language program (SCANERR) which calculates the value of δq_S for any combination of input values to eqns. (9)–(13). This program can be used to explore the error surface and find a minimum condition. As an example of such a calculation, Fig. 2 shows plots of δq_S as a function of scan rate for four different choices of sample mass, reference heat capacity and metabolic rate. Data from Table 1 were used in this calculation. Figure 2 shows a minimum error for our calorimetric system at scan rates of about 5 K h^{-1}. The error rises sharply at scan rates below 2 K h^{-1}. Scan rates above about 20 K h^{-1} were impractical because the calorimeter used requires about 30 min after the start of a scan to achieve a steady state condition where the Tian correction and associated errors are negligible.

One parameter which sometimes has an overriding influence on the design of an experiment is the amount of oxygen available for aerobic metabolism. In general, oxygen must not be depleted during the course of the measurements. An estimate of the time an experiment can run before oxygen depletion occurs, t_{ox} in seconds, can be obtained from eqn. (14):

$$t_{ox} = V_g f_g(273/T_g)(P_g/1\text{ atm})(1\text{ mmol}/22.4\text{ ml}) \times (467 \times 10^6\ \mu\text{J/mmol O}_2)/q_M \quad (14)$$

TABLE 1

Values of quantities used for error analysis and determination of metabolic rates of plant tissues by scanning temperature with a Hart Scientific DSC

Error in measured quantity	Estimated maximum value
δP_S	10 μW
δP_B	10 μW
$\delta \Delta T$	2 mK
$\delta \Delta t$	20 ms
δW_S	0.5 mg
δC_W	50 μJ mg^{-1} K^{-1}
δW_{Cu}	< 0.5 mg
δC_{Cu}	< 0.1 μJ mg^{-1} K^{-1}
Measured quantity	**Typical range of values**
b	1–10 K h^{-1}
$P_B/b_B = (C_A - C_R)$	−0.1–+0.1 J K^{-1}
$P_S/b_S = (C_A + C_S - C_R - C_C)$	0–2 J K^{-1} without Cu slug
	−1–+1 J K^{-1} with Cu slug
Δt	30–300 s
W_S	20–500 mg
q_S/W_S	0–5 μW mg^{-1}
C_W	≈ 4000 μJ mg^{-1} K^{-1}
C_{Cu}	≈ 380 μJ mg^{-1} K^{-1}
W_{Cu}	0–5 g

where V_g is the volume in milliliters of gas over the sample in the ampule, f_g is the volume fraction of oxygen in the gas, T_g is the Kelvin temperature of the gas used to fill the ampule, P_g is the pressure of the gas in the ampule in atmospheres, and q_M is the metabolic heat rate (μW). The last numerical

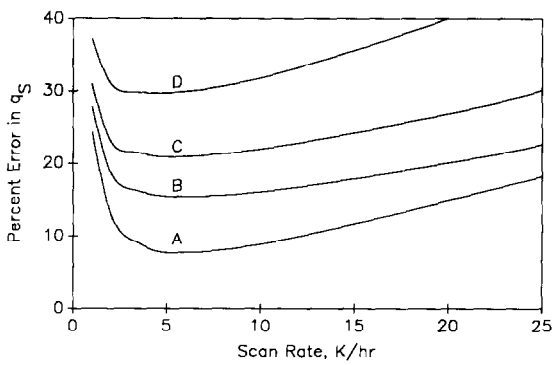

Fig. 2. Plots of the expected maximum error in q_S as a function of scan rate, sample mass, reference heat capacity, and metabolic heat rates: curve A, 250 mg sample at 2 μW mg^{-1} with 2 g of Cu; curve B, 250 mg sample at 2 μW mg^{-1} with no Cu; curve C, 50 mg sample at 2 μW mg^{-1} with no Cu; and curve D, 250 mg sample at 1 μW mg^{-1} with no Cu.

term in eqn. (14) is the heat of reaction per mole of O_2 with glucose. Use of this equation assumes that the heat of metabolism per mole of O_2 from all sources is not greatly different from the heat of oxidation of carbohydrate.

EXPERIMENTAL

Calorimeter

A Hart Scientific model 7707 heat conduction DSC was used in this study. This calorimeter has three sample chambers and one reference chamber. Three samples can be simultaneously subjected to the same temperature program. The samples need not be identical and it would usually be impractical to attempt to make them identical with many biological samples. Sample containers are 1 cm^3, 1 cm in diameter, cylindrical, thin-walled vials made from Hastelloy C and are readily removed from the calorimeter for cleaning, sterilization and filling. The screw caps are sealed with a flat washer made of Viton rubber. The calorimeter has a usable temperature range of -50 to $+100\,^{\circ}C$. The time constant varies somewhat with sample size, but is about 150 s with the samples used in this study. The scan rate is not constant; instead the temperature changes in a parabolic fashion, with the degree of curvature adjustable with switches on the calorimeter controller. Typically, a range of only about 30 K is scanned in the experiments described here. A non-linear scan may be used to advantage; the calorimeter can be adjusted to scan faster through temperature ranges of less interest. With proper choice of experimental variables and data analysis, no significant error is introduced by the non-linear scan rate.

A Haake model F3 circulating bath containing commercial ethylene glycol automotive antifreeze was used to circulate fluid through the calorimeter. Temperature fluctuations in the Haake bath were found to be of the order of ± 0.05 K. Baseline stability of the calorimeter could be improved with a better-regulated fluid bath.

Data were collected on an IBM PC–XT computer using the XENIX operating system supplied with the calorimeter.

Procedures and calculations

The computer programs and calibration constants supplied with the calorimeter were used to collect data, convert voltage data to μW data (P_B and P_S), and copy the resulting files containing P_B or P_S, T, and t values to ASCII files on a floppy disk. The ASCII files were then read into a BASIC program (FIXSUBCP) to reduce the data to values of P/b (see eqn. (6)) and subtract baseline data. These files of T, P_C, and b data were then used to compute q_S values in another BASIC program (METHEAT). Values for C_S

and C_C were entered into the METHEAT program as polynomials of temperature. Values of b were computed in the FIXSUBCP program from the temperature and time data, using eqn. (15):

$$b_n = 0.5[(T_{n+1} - T_n)/(t_{n+1} - t_n) + (T_n - T_{n-1})/(t_n - t_{n-1})] \quad (15)$$

which reduced to eqn. (16) because the data were taken at equal intervals:

$$b_n = (T_{n+1} - T_{n-1})/2\Delta t \quad (16)$$

In those experiments where C_C was not zero, a 2 g piece of copper was placed in the reference ampule and C_C was calculated from data in ref. 9 by fitting those data from 0 to 27 °C to a quadratic equation in temperature.

All regression analyses were done with Sigma Plot (copyright Jandel Corporation). Appendixes B and C contain the BASIC programs FIXSUBCP and METHEAT.

Materials

Barley (*Hordeum vulgare* CM72) seed was surface-sterilized for 15 min with 10% chlorox and sprouted between filter paper sheets wetted with one-quarter strength Hoagland's solution. Root tips ($\approx$ 1 cm) were removed with a razor blade 3–7 days after sprouting. About 100–200 mg of root tips were partially immersed in 200 µl of solution in the ampule. Tomato cells [10] were grown in modified Murashige and Skoog media [11], centrifuged, and transferred to sterile ampules in a laminar flow hood. About 150 mg of cells, wetted with about 50 µl of media, were placed in the ampule. The ampules were flushed with pure oxygen in order to avoid oxygen depletion during lengthy experiments. (Numerous experiments have shown that increased oxygen concentration in the gas phase does not affect the results obtained.) The culture was microscopically examined for microorganisms after each experiment. DEPC was obtained from Avanti Polar Lipids as a chloroform solution. The chloroform was evaporated with an argon stream and a 1 h lyophilization, aqueous buffer (0.1 M NaCl, 0.01 M MOPS, 0.1 mM EDTA, 0.02% NaN_3, adjusted to pH 7.6 with NaOH) was added, and a brief sonication gave the multi-lamellar suspension used in the calorimeter.

RESULTS

The results from one set of four test-scans with only water present in the ampules are given in Table 2. Since q_S must be zero for this experiment, any deviation from zero actually represents the error in the measurement at each data point. The results are in agreement with predictions from eqn. (9) and data in Table 1. (See also Fig. 2.) It is apparent from these results that propagation of small errors leads to unacceptably large errors in the result,

TABLE 2

Results of test scans with water in the measuring ampules and 2.0165 g Cu in the reference ampule: the data were analyzed using eqn. (8) with $C_S = 4183$ μJ K^{-1}

Scan	q_S (μW)	T(°C)	dq_S/dT (μW °C^{-1})	Amount of H_2O (mg)
Down	18	25	1.25	98.9
	−7	5		
	55	25	1.80	246.1
	19	5		
	63	25	3.30	487.9
	−3	5		
Up	3	25	0.60	98.9
	−9	5		
	−8	25	2.65	246.1
	−61	5		
	−25	25	2.65	487.9
	−78	5		
Up	−7	25	0.40	101.6
	−1	40		
	3	25	1.13	243.8
	20	40		
	5	25	2.80	485.9
	47	40		
Down	−12	25	1.53	101.6
	11	40		
	−53	25	1.47	243.8
	−31	40		
	−68	25	3.00	485.9
	−23	40		

q_S. This difficulty was overcome by measurement of q_S under isothermal conditions at temperatures at both ends of the scan, calculating the apparent values of C_S at these temperatures from eqn. (6), expressing C_S as a linear function of T, and using the resulting calculated C_S values to analyze the scanning data (see section on data analysis). The METHEAT program in Appendix C allows selection of the coefficients of the C_S function by successive approximations, which proved to be a reasonably efficient procedure.

Figure 3 shows the same data set used to generate Table 2 after the value of C_S had been chosen as a temperature-independent constant so as to give $q_S \approx 0$ at 24° C. As implied in Table 2, the scanning data are well described by a linear function of temperature. The unsmoothed data shown in Fig. 3 clearly show the magnitude of the errors between adjacent data points. Both point-to-point relative errors and slope errors are proportional to $(C_S - C_C)$.

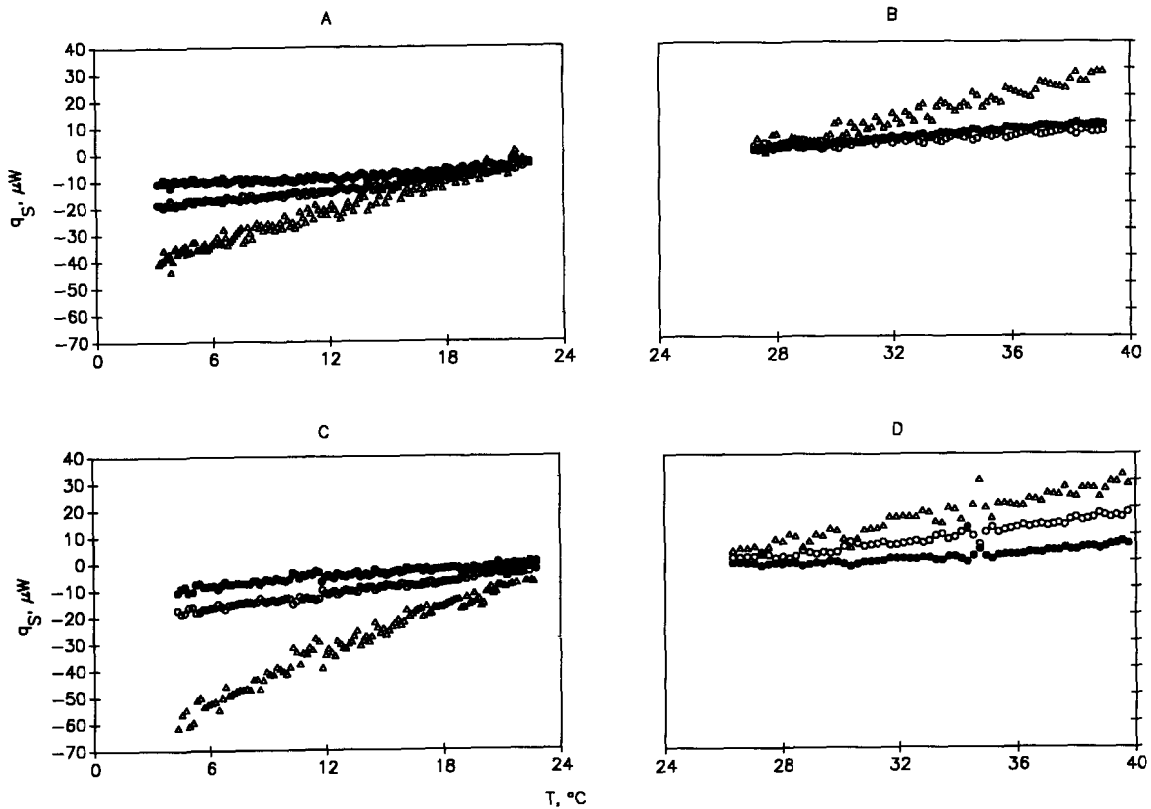

Fig. 3. Results of test runs with approximately 100, (●), 250 (○), and 500 (△) mg of H_2O in the sample ampules. The reference ampule contained a 2 g piece of copper. The scan rates were: A, −3.6 to −2.2° C h^{-1}; B, −3.6 to −2.5° C h^{-1}; C, 3.6–2.2° C h^{-1}; and D, 5.0–2.2° C h^{-1}. Values of q_S were obtained by adjusting C_S to give $q_S \approx 0$ at 24° C, as explained in the text. (See also Table 2.)

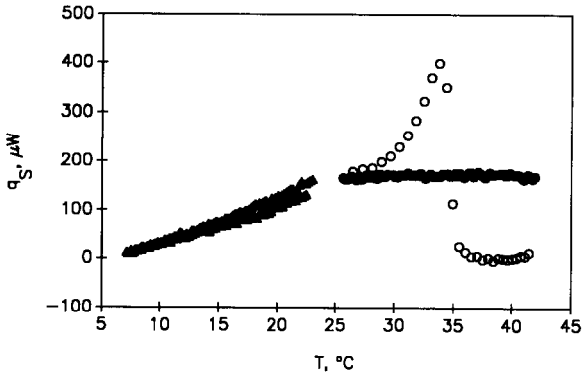

Fig. 4. Metabolic heat rate determined from temperature scans of barley root tissues. The samples consisted of root tips from 3-day old seedlings immersed in 200 μl of 1/4 Hoagland's solution. Scan rates and root weights were 2.2–0.8 °C h^{-1} and 445 mg for the slow scan from 24 to 42 °C (○), 6.9–5.5 °C h^{-1} and 327 mg for the faster scan from 24 to 42 °C (●), −3.8 to −2.5 °C h^{-1} and 356 mg for the scan from 24 to 7 °C (▲), and 3.0–1.8 °C h^{-1} for the scan from 7 to 23 °C (△).

Both ends of the curves shown in Fig. 3 could be adjusted to go through zero by expressing C_S as a linear function of T. This simply rotates the curves to a slope of zero without changing the shape. In the light of these results, we have chosen always to express C_S as a linear function of T in our subsequent studies of plant samples. Also, we have found that many plant tissues have a heat capacity very close to that of water.

It is of interest to note here that a similar correction procedure could be used to obtain accurate C_S values for non-thermogenic, i.e. $q_S = 0$, samples of unknown heat capacity as a function of temperature by DSC. The two known fixed-endpoint q_S values are equal to zero, so establishment of isothermal baseline data on both ends of a temperature scan allows accurate correction of the data for the minor experimental errors which normally lead to significant error in the calculated C_S values.

Figure 4 shows the metabolic heat rates of barley root tips over the temperature range from 5 to 40 °C determined by the scanning method. Below 25 °C the metabolic rate followed Arrhenius kinetics. Metabolic heat rate values obtained by isothermal and scanning methods are identical. This means that the scanning method measures a near steady state metabolic rate of the tissue.

Above 25 °C, metabolic heat rate measurements deviated from Arrhenius behavior. A steep increase in slope of the rate vs. T curve was noted between 30 and about 34 °C. Above 34 °C the heat rate declined to zero, indicating an upper crucial temperature at which metabolism stops. The measured heat rates above 30 °C were dependent on the scan rate. Increasing scan rates moved the onset of non-Arrhenius behavior and inactivation temperature to higher values. Steady state metabolic heat rates are not being measured at

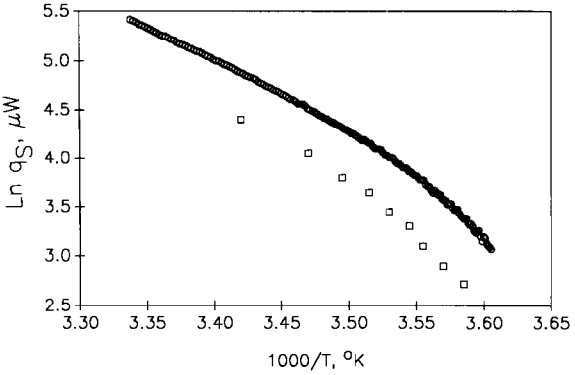

Fig. 5. Metabolic heat rates of tomato cells in tissue culture as a function of temperature plotted in Arrhenius form. Different cell samples were used for the scan (○) (419 mg scanned at -2.5 to -1.8°C h^{-1}), and for the isothermal measurements (□) (298 mg). All the isothermal measurements were made on a single sample, with temperatures changed stepwise without removing the ampule from the calorimeter.

the higher temperatures. Measurements, however, yielded consistent values for changes in temperature dependence and for inactivation temperatures in more than 20 scans of barley roots.

No indications of thermal transitions are apparent in the barley scans, though a transition could be masked by changes near 30 and 34°C. Consequently, an additional scan was done on a sample of barley roots treated with azide immediately prior to calorimetric examination. The metabolic heat rate of this tissue is near zero and could not mask thermal transitions. Still, no indication of thermal transitions was observed.

Unlike barley, tomato cells do not show an abrupt change in metabolic activity at some upper crucial temperature. Instead, a gradual decrease in the metabolic rate at temperatures above the upper crucial temperature is observed [5]. At temperatures below about 11°C, the metabolic rate of tomato cells drops below an Arrhenius extrapolation of metabolic rates above 11°C (Fig. 5). The low absolute values of the metabolic rate of tomato cells at low temperatures requires careful selection of experimental conditions for scanning experiments. However, data obtained by both scanning and isothermal methods yield identical conclusions.

Repeated isothermal measurements of metabolic heat rates at different temperatures are better than scanning for quantifying changes in slopes of q vs. T. However, the scanning method more clearly defines the time course and how abrupt the changes are. In addition, the scanning method allows the simultaneous observation of phase changes associated with changes in metabolic rates when phase changes are present.

To date, we have seen no heat effects in any of our data on living tissues which could be interpreted as lipid phase changes. Therefore, scans were run on pure lipids (see Fig. 6) to estimate our detection limit for phase changes.

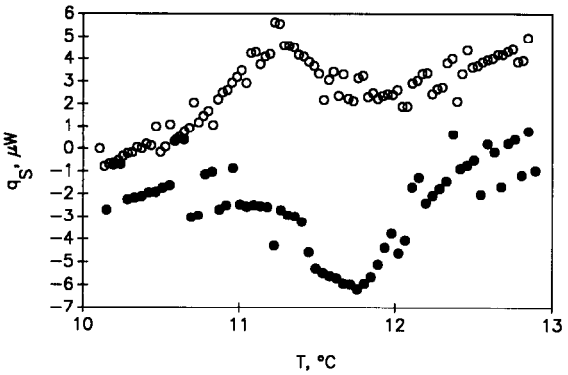

Fig. 6. Downward (○) and upward (●) scans across a reversible phase transition. The sample consisted of 0.1 mg of DEPC as a multi-lamellar suspension in aqueous buffer. The downward scan rate was $-3.6\,^{\circ}\mathrm{C\ h^{-1}}$ and the upward scan rate was $5.3\,^{\circ}\mathrm{C\ h^{-1}}$.

These data indicate that we should easily detect a lipid phase change involving more than about 0.1 mg of lipid over a range spanning less than 1° C. The shape of the peaks shown in Fig. 6 was essentially the same for scan rates from 2 to $30\,^{\circ}\mathrm{C\ h^{-1}}$ and for scans in which the lipid was combined with a tomato cell culture, either by mixing directly or by addition of lipid contained in a capillary tube.

For the actual cases of barley root and tomato culture studied, the expected detection limit for phase transitions, D_L, expressed as the fraction of lipid that could be seen, can be estimated for various conditions by approximating the peak by an isosceles triangle, assuming the width of the peak, ΔT_t, must be less than the range of temperature over which the observed change in the metabolic rate occurs, and assuming the height of the peak, h, must be greater than the baseline noise. Equation (17) expresses this relation where L is the amount of lipid present in the sample and ΔH is the heat effect of the phase transition:

$$D_L = (h\Delta T_t/b)/(L\Delta H) \tag{17}$$

The plant materials we have used in this study are about 1% lipid by weight. About 200 mg samples were used, and the ΔH value for the lipid phase transition is expected to be about 16 J g^{-1} [12]. Most of the changes in metabolic rate that we have observed occur in less than a 1° C span of temperature. Figure 6 shows that the peak-to-peak baseline noise is about 2.5 μW. The scan rates used in this study ranged from about 2 to $5\,^{\circ}\mathrm{C\ h^{-1}}$ although some experiments, carried out expressly to look for lipid phase transitions, were done at rates up to $20\,^{\circ}\mathrm{C\ h^{-1}}$. Therefore, we conclude that for most of our determinations, lipid phase transitions would have been observed if they occurred simultaneously in more than about 6–12% of the cell lipids.

DISCUSSION

Measurements of metabolic rates of plant tissues under a variety of temperature or other stress conditions are important for understanding the mechanisms of plant responses and for selection of tolerant or sensitive strains. Calorimetric measurements of metabolic heat rates are good indications of plant metabolic rates and can be easily used to monitor responses to changes under isothermal conditions. Measurements made while scanning over a wide temperature range have not previously been useful because no practical method for obtaining accurate q_S values was available, and because methods were not developed for separating the heat effects of metabolism from thermal events such as phase transitions. This scanning method solves these problems and now allows precise determination of temperature points which are crucial to metabolism as well as determination of how the metabolic activity changes with temperature between the critical temperatures. A DSC used for this type of study, however, must have sterilizable sample containers with a volume of about 1 cm^3 or greater, must be capable of scanning both upward and downward at very slow rates and, for some types of samples, i.e. roots, leaves etc., it is necessary to have a wide opening on the sample container.

A wet weight of 100–200 mg of cultured plant cells or tissue sections gives an adequate metabolic heat rate for scanning measurements. This amount of tissue also contains sufficient lipids in membranes so that phase transitions involving about 6% or more of this total lipid may be detectable.

Among other applications, we have successfully used the temperature scanning method to characterize the effects of changing temperature on tomato cell cultures, barley root tips [13], two species of redwood [14], larch [15], two species of magnolia, barley leaf tissue, wheat root tips, and corn root tips.

ACKNOWLEDGEMENT

L.D.H. thanks Hart Scientific, Inc. for a grant in partial support of this work.

APPENDIX A: BASIC PROGRAM SCANERR

```
20  CLEAR
30  CLS
35  PRINT "SCANERR"
40  PRINT "ERROR ANALYSIS OF SCANNING METHOD"
50  INPUT "SCAN RATE, K/HR ="; B
51  B = B/3600 : BB = B
```

```
 60  INPUT "TIME INTERVAL, sec = "; TI
 70  INPUT "SAMPLE WEIGHT, mg = "; W
 80  INPUT "METABOLIC HEAT RATE, uW/mg = "; Q
 90  INPUT "WT. OF Cu, mg = "; WCU
100  DPS = 10 : DPB = 10 : DDT = . 002 : DDTI = .02
110  DWS = .5 : DCW = 50 : DWC = .5 : DCC = .1
120  CCU = 380
199  REM : EQUATION 13
200  ECC = ((CCU * DWC) ^ 2 + (WCU * DCC) ^ 2) ^ .5
210  IF WCU = 0 THEN ECC = 0
219  REM : EQUATION 12
220  ECS = ((4183 * DWS) ^ 2 + (W * DCW) ^ 2) ^ .5
229  REM : EQUATION 11
230  EB = ((DDT/TI) ^ 2 + ((B/TI) ^ 2) * DDTI * DDTI) ^ .5
239  REM : EQUATION 10
240  X1 = (DPS/B) ^ 2
245  X2 = (DPB/BB) ^ 2
250  X3 = 4183 * W - 380 * WCU + .1
255  IF X3 < 0 THEN X3 = X3 - .2
256  X3 = X3 * X3
260  X4 = (EB/B) ^ 2
265  X5 = (.1/BB) ^ 2
269  REM : ASSUME EBB = EBS = EB
270  EPC = (X1 + X2 + X3 * X4 + X5 * X4) ^ .5
279  REM : EQUATION 9
280  QS = Q * W
290  EQS = B * B * (EPC * EPC + ECS * ECS + ECC * ECC)
300  EQS = EQS + ((QS/B) ^ 2) * EB * EB
310  EQS = EQS ^ .5
320  B = B * 3600
400  LPRINT "SCAN RATE = "B; "K/HR"
410  LPRINT "TIME INTERVAL = "; TI; "secs"
420  LPRINT "SAMPLE WEIGHT = "; W; "mg"
430  LPRINT "MET. HEAT RATE = "; Q; "uW/mg"; QS; "uW"
440  LPRINT "WT. OF CU = "; WCU; "mg"
450  LPRINT " "
460  LPRINT "ERROR IN REF. HEAT CAP. = "; ECC; "uJ/K"
470  LPRINT "ERROR IN SAMPLE HEAT CAP. = "; ECS; "uJ/K"
479  EB = EB * 3600
480  LPRINT "ERROR IN SCAN RATE = "; EB; "K/HR"
490  LPRINT "ERROR IN PC = "; EPC; "UJ/K"
500  LPRINT "DPS/B, DPB/BB, (PS/B)(EB/B), (PB/BB)(EB/B)"
510  LPRINT X1, X2, X3 * X4, X5 * X4
520  LPRINT "ERROR IN QS = "; EQS; "uW"
530  LPRINT " "
540  LPRINT " "
550  LPRINT " "
560  LPRINT " "
570  GOTO 20
```

APPENDIX B: BASIC PROGRAM FIXSUBCP

```
20  CLEAR
30  CLS
34  IF M=1 GOTO 50
35  M=0
39  PRINT "FIXSUBCP"
40  PRINT "PROGRAM TO CONVERT DSC.H* FILES TO DSC.CP* FILES AND
    SUBTRACT BASELINE FILES"
41  PRINT "BASELINE FILES OF PROPER FORM ARE ALSO GENERATED BY
    THIS PROGRAM"
45  DIM T(1500), Q(1500), TI(1500), B(1500), C(1500), TB(1500), CB(1500), A(10)
46  M=1
50  INPUT "INPUT FILENAME (TYPE Q TO QUIT)"; IN$
60  IF IN$="Q" THEN END
65  INPUT="OUTPUT FILENAME"; OT$
70  OPEN IN$ FOR INPUT AS #1
80  INPUT "START TEMPERATURE", TS
90  INPUT "END TEMPERATURE", TE
95  INPUT "DATA POINT INTERVAL", NS
100 J=0
110 J=J+1:N=NS
120 JX=J
130 INPUT #1, T(J), Q(J), TI(J), X:PRINT T(J), Q(J), TI(J), J
135 IF EOF(1) THEN GOTO 200
140 IF TS<TE GOTO 185:REM:UPSCANS
150 IF T(J)>TS GOTO 130
155 N=N-1:IF N>0 GOTO 130
160 IF T(J)>TE GOTO 110
170 GOTO 200
185 IF T(J)<TS GOTO 130
187 N=N-1:If N>0 GOTO 130
190 IF T(J)<TE GOTO 110
200 CLOSE #1
210 FOR J=2 TO JX-1
220 B(J)=(T(J+1)-T(J-1))/(TI(J+1)-TI(J-1))
230 C(J)=Q(J)/B(J)
240 NEXT J
300 INPUT "DO YOU HAVE A BASELINE FILE (Y/N)"; X$
310 IF X$="N" GOTO 900
320 INPUT "BASELINE FILENAME"; IB$
330 OPEN IB$ FOR INPUT AS #1
360 INPUT #1, A(0), A(1), A(2), A(3), A(4), A(5), A(6), A(7), A(8), A(9)
390 FOR J=2 TO JX-1
395 FOR N=0 TO 9
400 C(J)=C(J)-(A(N)*T(J)^N)
410 NEXT N
420 NEXT J
900 OPEN "O", #2, OT$
905 FOR J=2 TO JX-1
910 WRITE #2, T(J), C(J), B(J)
915 NEXT J
```

```
 917  CLOSE #2
 920  LPRINT IN$; "OUTPUT TO"; OT$
 930  LPRINT "START TEMP. ="; TS; "C"
 940  LPRINT "END TEMP. ="; TE; "C"
 945  IF X$ = "N" THEN IB$ = "NOT"
 950  LPRINT "BASELINE FILE"; IB$; "SUBTRACTED"
 960  GOTO 20
 980  PRINT "BASELINE FILE OUT OF RANGE"
 990  GOTO 20
1000  END
```

APPENDIX C: BASIC PROGRAM METHEAT

```
 20  CLEAR
 25  I = 0
 30  CLS
 35  PRINT"          METHEAT
 36  PRINT
 40  PRINT "PROGRAM TO COMPUTE METABOLIC HEAT RATE FROM DOS DSC
     DATA FILES OF", "THE FORM TEMPERATURE, HEAT CAPACITY, TIME.
     THE t MUST BE REMOVED FROM THE TIME", "COLUMN IN XENIX FILES"
 50  PRINT
 60  PRINT "YOU WILL NEED TO ENTER THE VALUES OF THE COEFFICIENTS
     IN POLYNOMIAL", "EQUATIONS FOR THE SAMPLE AND REFERENCE",
     "HEAT CAPACITIES AS A FUNCTION OF TEMPERATURE."
 61  PRINT
 63  PRINT "FILENAMES ARE ENTERED AS quoteX:FILENAMEquote WHERE X
     IS THE DEVICE,", "i.e., A, B, OR C."
 65  DIM A(10), B(10), T(1530), PC(1530), TI(1530), QS(1530), QSS(1530), LQS(1530),
     LQSS(1530), RTK(1530)
 70  INPUT "INPUT FILENAME (TYPE Q TO QUIT) "; IN$
 71  IF IN$ = "Q" THEN END
 75  PRINT "OPENING FILE", IN$
 80  INPUT "OUTPUT FILENAME"; OT$
 85  PRINT "OPENING OUTPUT FILE"; OT$
 86  INPUT "START TEMPERATURE ", TS
 87  INPUT "END TEMPERATURE", TE
 88  OPEN IN$ FOR INPUT AS #1
 89  J = 0
 90  J = J+1
 91  JX = J
 92  INPUT #1, T(J), PC(J), TI(J):PRINT T(J), PC(J), TI(J), J-1
 93  IF TS < TE GOTO 97:REM:UPSCANS
 94  IF T(J) > TS GOTO 92
 95  IF T(J) > TE GOTO 90
 96  GOTO 99
 97  IF T(J) < TS GOTO 92
 98  IF T(J) < TE GOTO 90
 99  CLOSE #1:LA$ = "N":L$ = "Y"
```

```
100 PRINT "COEFFICIENTS FOR REFERENCE HEAT CAPACITY EQUATION,
    uJ/K"
101 IF I = 0 GOTO 110
102 PRINT "THE CURRENT VALUES OF THE COEFFICIENTS ARE"
103 FOR J = 0 TO N: PRINT "B("; J; ") ="; B(J): NEXT J
104 INPUT "DO YOU WANT TO CHANGE THE B VALUES? (Y/N) ", L$
105 IF L$ = "N" GOTO 160
110 INPUT "NUMBER OF COEFFICIENTS = "; N
111 IF N > 10 THEN PRINT "MAX N = 10": GOTO 110
120 J = -1
130 J = J + 1
140 PRINT "B("; J; ") = "; : INPUT B(J)
150 IF J < N - 1 GOTO 130
160 PRINT "COEFFICIENTS FOR SAMPLE HEAT CAPACITY EQUATION,
    uJ/K/mg"
161 IF I = 0 GOTO 170: PRINT "THE CURRENT VALUES ARE"
162 FOR J = 0 TO M: PRINT "A("; J; ") ="; A(J): NEXT J
163 IF LA$ = "Y" GOTO 200
164 INPUT "DO YOU WANT TO CHANGE THE A VALUES? (Y/N)", L$
165 IF L$ = "N" GOTO 212
170 INPUT "NUMBER OF COEFFICIENTS = "; M
171 IF M > 10 THEN PRINT "MAX M = 10": GOTO 170
180 FOR J = 0 TO (M - 1): PRINT "A("; J; ") = "; : INPUT A(J): NEXT J
185 GOTO 211
200 FOR J = 0 TO (M - 1): PRINT "A("; J; ") = "; : INPUT A(J): NEXT J
211 IF LA$ = "Y" GOTO 360
212 INPUT "TOTAL SAMPLE WEIGHT IN AMPULE = "; SW
214 INPUT "WEIGHT OF TISSUE IN SAMPLE = "; TW
216 INPUT "DATA PT. INTERVAL = ", IXT
360 K = 2
365 Y = T(K)
380 CCU = 0: L = 0
390 CCU = CCU + B(L) * Y ^ L
400 L = L + 1
410 IF L < = N GOTO 390
430 CST = 0: L = 0
440 CST = CST + A(L) * Y ^ L * SW
450 L = L + 1
460 IF L < = M GOTO 440
463 QS(K) = (PC(K) + CST - CCU) * TI(K)
465 PRINT TI(K), QS(K), K
471 RTK(K) = 1000/(T(K) + 273.15)
472 QSS(K) = QS(K)/TW
473 IF QS(K) = < 0 GOTO 478
474 LQS(K) = LOG(QS(K))
476 LQSS(K) = LOG(QSS(K))
477 GOTO 480
478 LQS(K) = -10
479 LQSS(K) = -10
480 K = K + IXT
490 IF K < JX GOTO 365
495 INPUT "SAVE DATA TO DISK AND PRINTER? (Y/N)"; L$
```

```
496 IF L$ = "N" GOTO 555
497 LPRINT IN$; "OUTPUT TO"; OT$
498 FOR J = 0 TO M: LPRINT "A("; J; ") = "; A(J); "uJ/K/mg sample": NEXT J
499 FOR J = 0 TO N: LPRINT "B("; J; ") = "; B(J); "uJ/K": NEXT J
500 OPEN"O", #2, OT$
501 LPRINT "START TEMP. = "; TS; " C": LPRINT "END TEMP."; TE; " C"
502 LPRINT "SAMPLE WT. = "; SW; " mg": LPRINT "TISSUE WT. = "; TW; " mg"
504 LPRINT "TOTAL NO. OF DATA POINTS IS"; JX; "POINTS USED - "; JX/IXT
505 LPRINT "T(START) = "; T(2); " QS(START) = "; QS(2); " uW"
506 LPRINT "T(END) = "; T(K - IXT); " QS(END) = "; QS(K - IXT); " uW"
507 LPRINT "SCAN RATE AT START = "; 3600 * TI(2); " K/HR"
508 LPRINT "SCAN RATE AT END = "; 3600 * TI(K - IXT); " K/HR"
510 J = 2
520 WRITE#2, T(J), QS(J), QSS(J), LQS(J), LQSS(J), RTK(J), TI(J)
530 J = J + IXT
540 IF J < JX GOTO 520
550 CLOSE #2
555 I = 1
556 INPUT "RECYCLE WITH NEW A(J) VALUES? (Y/N)"; LA$
557 IF LA$ = "Y" GOTO 161
560 GOTO 70
570 END
```

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