

Effects of temperature and oxygen depletion on metabolic rates of tomato and carrot cell cultures and cuttings measured by calorimetry

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Abstract. Isothermal heat-conductance calorimetry was used to monitor responses of tomato and carrot metabolism to changes in temperature and oxygen concentrations. Calorimetric measurements of metabolic heat evolution from tissue segments and cultured cells was found to be a sensitive, non-destructive estimate of metabolic rates. Short-term measurements of metabolic rates of cells in culture correlate well with calorimetric measurements made on tissue sections. The results accurately predict the growth properties of intact plants based on the generally recognized characteristics of these two species. The calorimetric method provides another means for rapid evaluation of plant responses to physical and chemical stresses and is of value for screening and selection.

Key-words: Calorimetry; chilling; heat-shock; metabolic rates; temperature sensitivity; carrot; tomato; oxygen.

Introduction

Metabolic rates of plants are related directly to the rate at which a plant can grow; i.e., a plant cannot grow rapidly unless it metabolizes rapidly. Under conditions which are detrimental to growth, metabolism will either be slowed or will become more inefficient at producing growth. Measurement of metabolic rates of plants or tissues can, therefore, provide a general measure of plant responses to changes in environment and appears to be a reasonably good indicator of plant growth rates.

The rates of metabolism of plants can be determined by three basically different methods. One is to determine the biomass produced over a given time. This may be a lengthy, costly, and generally difficult procedure often requiring extended control of many variables influencing plant growth and complicated statistical analysis. It also requires growth of many plants, since an individual plant cannot be used as its own control. A second method is to use some biochemical measure such as the rate of O_2 uptake, CO_2 release, or the rate of incorporation of some labelled

nutrient. Such methods have proven effective, but may not reflect changes or responses which happen to affect a part of plant metabolism not included in the particular metabolic cycle measured. A third method of measuring metabolism is to measure rates of heat evolution. Heat evolution from cells in a steady-state condition is interpretable as a measurement of overall metabolic rate (Loike, Silverstein & Sturtevant, 1981). Stress conditions such as extremes in temperature, decreased oxygen tension, increased salinity or added inhibitors cause changes in metabolic rates which can be monitored by changes in heat evolution. (Unpublished data from this laboratory.) Until recently, measurements of heat production by plant tissues have been limited by available instrumentation (see for example Steward, 1940). Equipment currently available overcomes many of these limitations. It is now possible to determine rapidly metabolic heats of small samples of tissue, or cultured cells in suspension, over a wide range of experimental conditions. Microcalorimetric measurements of metabolic heat evolution from cells and tissue samples can provide sensitive and precise evaluations of tissue responses to varied environmental factors, chemical treatments or genetic alterations. The techniques should prove useful for genetic screening and selection.

We report here the use of isothermal heat conduction microcalorimetry as a rapid non-destructive method of monitoring cellular responses in parallel studies of both tissue sections and cultured cells of tomato and carrot. The metabolic properties of these samples were examined to test whether correlations exist between the effects of short-term temperature changes on the metabolic rates of the test materials and the generally recognized effects of temperature on the growth properties of these species. The relationship between heat evolution and the PO_2 was also examined.

We initially selected non-differentiating cells growing asynchronously in culture for this study, based on the postulate that the measured metabolic properties of these cultures would reflect general aspects of cell metabolism and would be independent of specific cell cycle or differentiation processes. Subsequently, differentiated tissue segments were also examined for

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comparison. The results show nearly identical responses of cultured cells and selected tissues and demonstrate that either can serve as a reasonable predictor of intact plant growth characteristics.

Materials and methods

Tomato cells were from a cell line developed by Dr. D. Pratt, Department of Bacteriology, U. C. Davis. This line was obtained from leaf explants of seedlings of a *Lycopersicon esculentum/L. peruvianum* hybrid 741505-45. This hybrid was produced and described by Hoogenboom (1972), and Pratt (personal communications). The cells are grown on modified Murashige and Skoog media (Thomas & Pratt, 1981). Carrot cells were a diploid line of wild carrot, *Daucus carota*, and were a generous gift from H. Bonnet, University of Oregon. Carrot cells were maintained on N12D medium (Erickson, 1965).

Tomato tissue samples were obtained from greenhouse grown *Lycopersicon esculentum/L. peruvianum* hybrid 741505-45. The terminal 2 cm of lateral shoots, including developing leaves were excised and placed directly in the calorimeter with 100 mm³ of the growth media cited above. Carrot tissue samples were obtained from carrots purchased at a local market. Tissue segments were prepared by boring plugs of root tissue perpendicular to the root axis with a cork borer. The tissue plugs, varying in dry weight from 150 to 300 mg, were placed in the calorimeter ampule with 100 mm³ growth media as above to maintain a saturated atmosphere in the ampoule with no desiccation of the sample.

Rates of metabolic heat production were measured with a Hart Scientific model 7707 calorimeter with isothermal and temperature scanning capabilities. (Hart Scientific Inc., 177 West 300 South, Provo, Utah 84601). This calorimeter measures the difference in the rates of heat flow between a constant temperature metal block and a reference ampule and a sample ampoule. The rate of heat flow is measured as proportional to the output voltage signal from a Seebeck device placed between each ampoule and the block. In this study a calorimeter with only one measuring and one reference cell was used. (The rate of sample throughput has been greatly increased in later studies by use of a calorimeter with multiple measuring cells.) Samples were placed in Hastelloy-C ampoules with an internal volume of 1.05 cm³. The calorimeter was calibrated electrically at 3.64 and 36.30 °C for the experiments of Table I and at 1.68, 4.65, 9.57, 14.50 and 23.31 °C for all other experiments. Values of the calibration constant at other temperatures were obtained by linear interpolation. Except for temperature adjustments between experimental points, the calorimeter was operated in the isothermal mode. An empty ampoule served as the reference.

Tomato cells and carrot cells growing in log phase on the sucrose carbon source were collected by pipetting and passed with low suction onto a Whatman

GFC filter cut to a diameter slightly smaller than that of the ampoules. Filter and cells were left saturated with culture medium and placed into the ampoules which were then inserted into the calorimeter sample compartments where the metabolic heat was measured. In most cases, a single sample of cells (3.5 to 5 mg dry weight) was used to investigate metabolic rates for each species over the entire temperature range studied. Following measurement of heat production at each temperature the ampoules with cell samples were removed, opened to admit air, and replaced. This procedure caused a small measurement uncertainty of about 10 µW because of baseline uncertainty. Each measurement took about one hour. Data were recorded at 10 s intervals using an IBM-XT computer. The apparent time constant of the calorimeter under these conditions is about 7 min, so the samples reached a steady state in less than 45 min. Measurements of metabolic rates for different samples of tomato or of carrot tissues were normalized by a linear scaling method for comparative purposes to allow all data on each tissue to be combined in a common Arrhenius plot. The rationale for this treatment of the data is developed in the discussion section below.

Results

Table I and Figs. 1 and 2 present data on the steady state heat production of tomato and carrot samples. Table I reports data collected during studies of a single sample of cultured tomato cells exposed to various temperatures during a 24 h period. The results at 23 °C (tests number 1, 4, and 10) demonstrate the reversibility of the responses after exposure of the cells to both higher and lower temperatures. These data, together with similar data accumulated for Figures 1 and 2, show that short-term temperature cycling of the cell cultures does not permanently affect the culture over the temperature range of 0 °C to 36 °C for tomato and 0 °C to 32 °C for carrot. Even when tomato cells were exposed to low temperatures, where metabolism and growth were negligible for greater than one hour, readjustment to a higher temperature reproducibly returned the cells to the same higher rate of metabolism. Neither a loss of viability of cells nor slowly reversible changes were detected.

The high degree of reproducibility of metabolic rate measurements after several hours of temperature cycling in a random fashion (s.d. = 9.6 µW) also rules out the possibility that we are observing effects of microbial contaminants, since the growth of microorganisms would give rise to large increases in heat production. No evidence for microbial contamination was found during the course of any of the measurements reported.

Figs. 1A and B show temperature effects on the rates of metabolism of cultured cells and tissue segments of tomatoes and carrots, respectively. The data

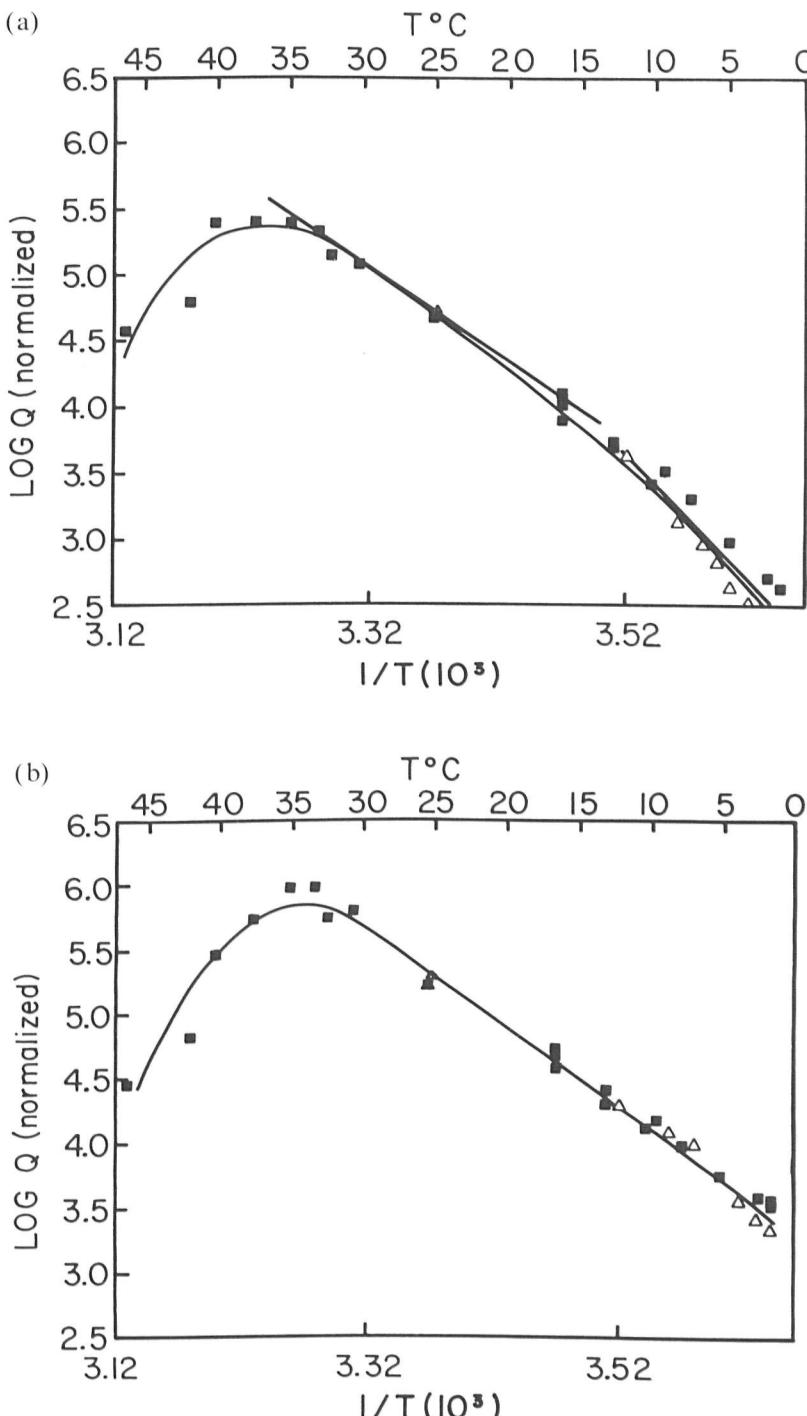


Figure 1. A. Log rate of heat release plotted vs. reciprocal temperature (degrees K) showing the effects of temperature on the metabolic rates of tomato cells (—■—■—) and tomato shoots (—△—△—). The cell data were collected from three separate cell cultures. The lines were fit by least square procedures to the points below 9.5 °C and in the range 9.5 to 32 °C.

B. Log rate of heat release plotted vs. reciprocal temperature (degrees K) showing the effects of temperature on the metabolic rates of carrot cells (—■—■—) and root tissue (—△—△—). The cell data were collected from three separate cell cultures. A line is fit by least square procedures to the data over the temperature range up to 32 °C.

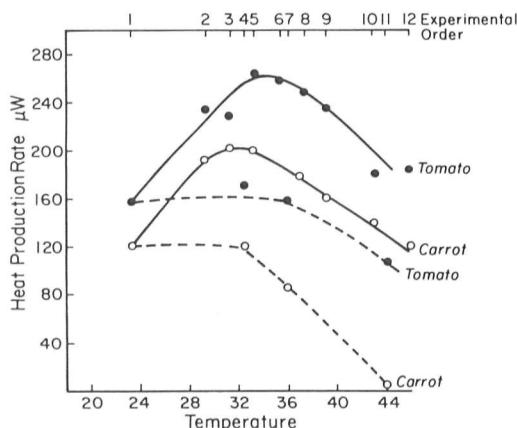


Figure 2. Calorimetric measurements of metabolic rates of tomato and carrot cell cultures at temperatures above 23 °C. The solid curves are measured heats of metabolism at a series of increasing temperatures taken in order (upper abscissa). Periodically during the series of measurements at increasing temperatures, the rate at 23 °C was remeasured. The dashed curves are the point at 23 °C plotted in the order taken relative to all points.

are plotted in Arrhenius form to illustrate discontinuities or abrupt changes in slope of the rate data at critical temperatures. Significant changes in slopes are seen to occur in both the high and low temperature ranges for tomatoes using either cultured tomato cells or tissue segments. The slope change noted in the 11 °C to 14 °C range for chilling sensitive tomato preparations is notably absent in the insensitive carrot tissue.

Figure 2 presents a further analysis of metabolic events in the high temperature range. Metabolic rate data are presented in two forms. The solid curves show directly the measured heat production rates of tomato and carrot cells at different temperatures in the range from 23 °C to 45 °C. The metabolic rates increase, plateau, and then decrease with increasing temperatures in this range. The dashed curves of Fig. 2 illustrate reversibility of metabolism following exposure of the samples to successively higher temperatures. All points on these curves measured metabolism at 23 °C for samples which had been

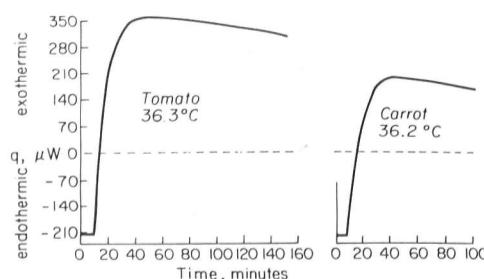


Figure 3. Metabolic rates of tomato cells measured as a function of time at 36 °C and 23 °C. Initial large changes in apparent values of q are caused by heating ($-q$) or cooling ($+q$) to adjust to thermal equilibrium. At 23 °C, the measured metabolic rate remained constant for the duration of experimental tests (lower curve). Rates at 36 °C decreased linearly with time (upper curve) and initial metabolic rates were determined by extrapolation to zero time at 36 °C.

Table 1. Heat production rates of (84 mg wet weight, 4.2 mg dry weight) undifferentiated tomato cell culture.

Test number	Time, hours ^a	Temperature, °C	Heat production rate, μW^b
1	1.0	23.1	165
2	2.0	12.4	51
3	2.8	15.3	70
4	4.1	23.1	147
5	5.1	3.6	2
6	6.2	10.4	24
7	7.1	20.1	107
8	7.9	30.2	252
9	8.0	36.3	378 ^c
10	11.0 ^d	23.1	143
11	12.1	23.1	143
12	12.7	23.1	139
13	13.5	23.1	132
14	14.3	23.1	119
15	15.2	23.1	95
16	15.5	23.1	84
17	15.7	23.1	68
18	15.8	23.1	46
19	16.0	23.1	24
20	16.2	23.1	13
21	16.3	23.1	8
22	16.5	23.1	6
23	16.8	23.1	4
24	23.5	23.1	4

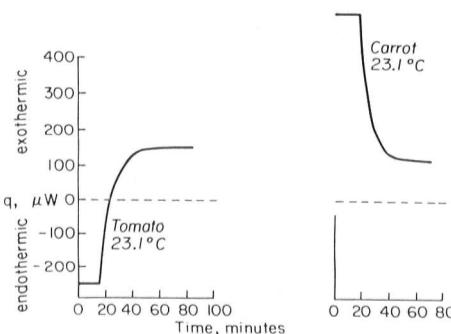
^aTime was set equal to zero when the cells were placed in the ampoule.

^bs.d. = 9.6 μW .

^cValue given is extrapolated from 9.5 to 8.0 h.

^dThe ampoule was left sealed and in the calorimeter for the remainder of the time.

previously exposed to higher temperatures. Experiments were run in the order indicated on the upper abscissa. For example, experiment #4 measured metabolic rates of carrot and tomato cells at 23 °C after they had been exposed to 31 °C in experiment #3. The data show that tomato cells exposed to temperatures greater than 36 °C and carrot cells exposed to temperatures greater than 32 °C suffered some reduction of metabolism that is either irreversible or slowly reversible when compared to the response below these temperatures.



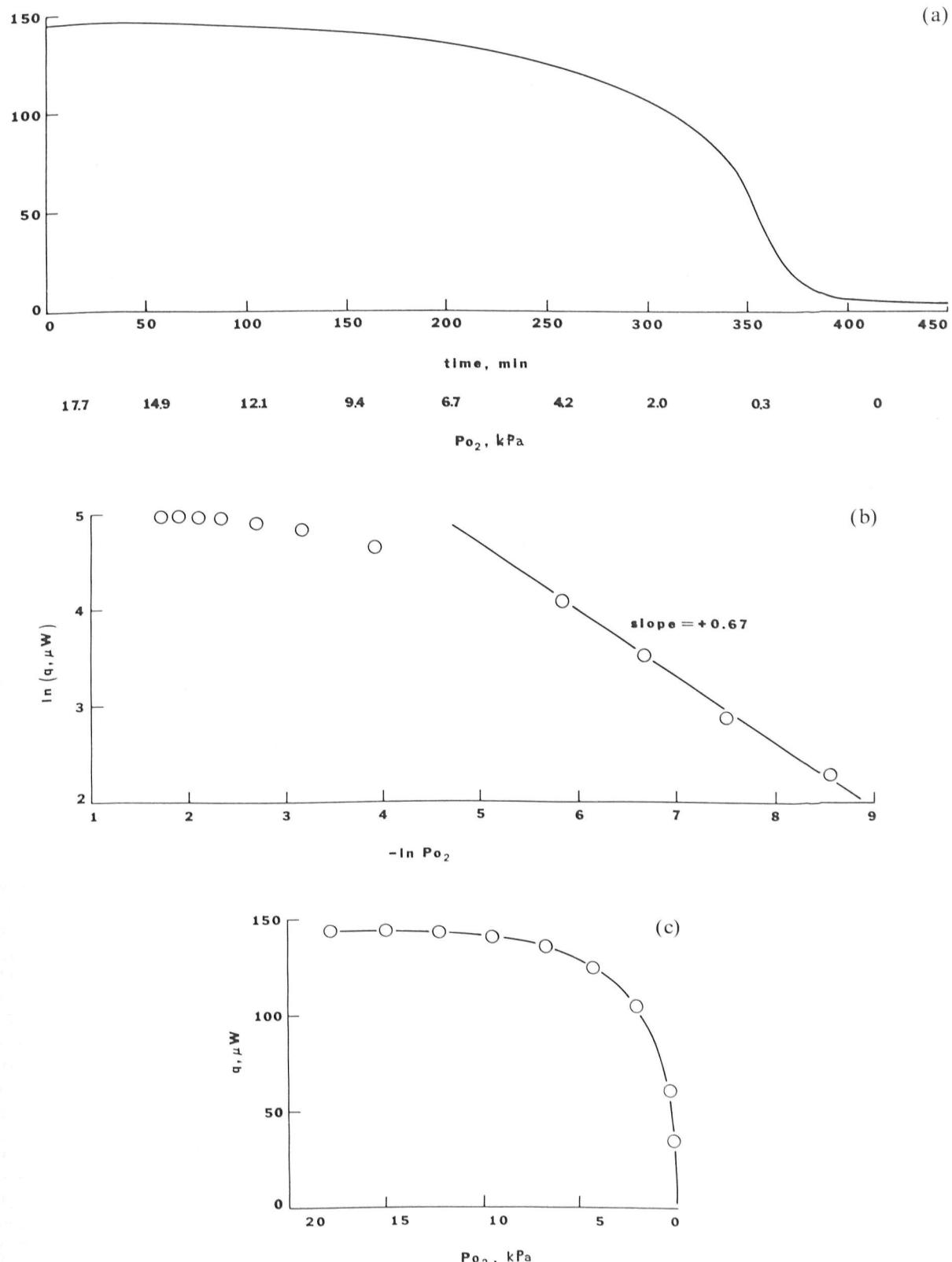


Figure 4. The effect of oxygen partial pressure on the metabolic rate of tomato cell cultures. The data are expressed as a function of time (A); as a linear function of $P(O_2)$ (B); and as the log function expressed in equation 7(C). The data are the 11 h to 23.5 h points of Table 1. Cells in the sealed sample ampoule were maintained at 23.1 °C and the metabolic rate was followed while oxygen was depleted metabolically from the ampoule.

Figure 3 shows the time courses of microcalorimetric measurements of heat rate, q . The initial large changes in measured values of q illustrate the time taken for the calorimeter ampoules to come to thermal equilibrium. The values, which represent the metabolic heat production rates from the samples, then stabilize. The direction of the initial change in observed values of q depended upon whether thermal equilibrium was approached from a higher or lower temperature in the previous test. The final value of q at a given temperature was independent of the direction from which it was approached, indicating the reversibility of the metabolic rates with temperature.

Metabolic heat rates remained remarkably constant for cell or tissue samples maintained at constant temperatures below the plateau regions in Fig. 2. However at temperatures in the ranges of 32 °C to 38 °C for tomatoes and 28 °C to 32 °C for carrots, the rates of heat production by the samples decreased linearly with time after the response of the calorimeter to the temperature change was completed (see Fig. 3 and compare with the 23.1 °C data of Fig. 4 showing nearly constant heat production for >100 min). Therefore, the heat production data at these higher temperatures in both tomato and carrot cells indicated that changes in metabolism were occurring at these temperatures. The data also illustrate the ability of the calorimetric method to quantitate the magnitude and time courses of such stress-induced changes in metabolism.

Figures 4A,B and C show the dependence of metabolic rates of tomato cells as a function of ${}^P\text{O}_2$ (see also tests numbered 10 to 24 of Table 1). The data are plotted in three forms to emphasize different features of the results. Figure 4A illustrates the long-term stability of the metabolic rate of tomatoes at 23.1 °C. The heat production rates were nearly constant for over 200 min while PO_2 remained above 6 to 7 kPa (see also Fig. 4C). Below this level, a decreasing rate was noted which was shown to depend on the 2/3 power of PO_2 concentration by the log plot of Fig. 4B. Finally Fig. 4C illustrates that relatively only minor decreases in metabolic rates were seen for reduction of PO_2 to values as low as 2 kPa.

When cells are incubated in an atmosphere with initially elevated O_2 levels in the ampoule, the metabolic heats are maintained at a constant value for a proportionally increased time before the final rapid decrease to zero (data not shown). This confirms that the rapid decrease noted in Fig. 4A is related to O_2 levels. Moreover, increasing cell mass in test samples reduces the time at which the departure from constant metabolic heat rate is observed. Again, this time of decrease is directly related to cell mass and heat rate.

Discussion

The rate of metabolic heat production, q , may be described mathematically in a rate expression of the

form:

$$q = (\Delta H)(r) = \Delta Hk[f(\text{conc.})\text{PO}_2^n + M] \quad (1)$$

where ΔH is the heat produced per unit of metabolic reaction; r is the metabolic rate; k is the rate constant; PO_2 is the partial pressure of oxygen; $f(\text{conc.})$ is a function of the concentrations of the growth media components; n is a constant at a given temperature and M represents any non-respiration linked heat producing (utilizing) events. If PO_2 and $f(\text{conc.})$ are held constant and M is small relative to respiratory heat, then

$$r = kC \quad (2)$$

where C is a constant. The temperature dependence of k may be described in the form of the Arrhenius equation

$$k = A e^{-E'/RT} \quad (3)$$

where A is an empirical constant, E' is the activation energy, R is the gas constant and T is the Kelvin temperature. Thus we have the temperature dependence of the rate as

$$r = CA e^{-E'/RT} \quad (4)$$

and, assuming that ΔH is constant over the temperature range, then

$$q = \Delta HCA e^{-E'/RT} \quad (5)$$

or in log form

$$\ln q = \ln[\Delta HCA] - E'/RT \quad (6)$$

which means that a plot of $\ln q$ against $(1/T)$ will be linear with a slope of $-E'/R$ if the assumptions and approximations are adequate. Figures 2 and 3 show the plots obtained from a number of experiments on both cells and tissues. The use of the logarithmic transformation in the Arrhenius calculation yields values of an extensive property with slopes independent of sample size. The intercept is a function of sample size, however. Therefore, data accumulated for separate samples were normalized at a reference temperature (23 °C). At the higher temperature ranges studied the function deviates from linearity and the slope eventually becomes negative. With tomato, the factor(s) causing the decreased slope was reversible up to 36 °C (note also Table 1). Above this temperature irreversible or slowly reversible metabolic changes occurred (note also Figure 2). Similar results were obtained with carrot above 32 °C.

At temperatures below 9.5 °C, the negative slope of the logarithmic function for tomato cells and tissues is increased, indicating a higher temperature coefficient by 1.8. This corresponds in Arrhenius theory to an

increase in activation energy from 54.8 to 98.7 kJ/mole (13.1 to 23.6 kcal/mole). For carrots any deviation at low temperature is small and, if present, is near 0 °C. The possible causes of such changes in E' may be a loss of activity of enzymes, structural changes, or altered rates of enzyme synthesis. Carrots must have metabolic pathways which are not as temperature sensitive as the tomato pathways in the low temperature range. Various postulates have been reviewed (Lyons, Graham, and Raison, 1979; Graham and Patterson, 1982).

The dependence of metabolic rate on PO_2 was investigated for tomato cells and the data were plotted in Fig. 4 as a function of time (A), as a linear function of PO_2 (B), and as the log form of equation 1 (C), i.e.

$$\ln q = \ln[\Delta H k f(\text{conc.})] + n \ln PO_2 \quad (7)$$

To evaluate values of PO_2 , it was assumed that the fraction of O_2 used was equal to the fraction of total heat produced and that PO_2 reached zero at 16.8 h into the experiment (see Table 1). At this point the ampoule, which initially contained 4.37 μmoles of O_2 , had been sealed for 6.9 h. The value of n found from the low PO_2 end of Fig. 4B is 0.67. The other interesting result from Fig. 4 (B and C) is that q , and hence the metabolic rate, decreases only slightly until the calculated $PO_2 < \text{kPa}$.

The assumption that limiting O_2 was responsible for the rapid decrease in heat, seen near 300 min in Fig. 4, was tested by performing experiments at higher initial levels of O_2 and also with larger cell masses. The time to deplete O_2 was directly proportional to the amount of O_2 in the ampoule and inversely proportional to the cell mass. Together, these tests rule out a decrease caused by some undetermined, mass-related property of the cells or by buildup of metabolic products in the cells. A value of ΔH can also be obtained from the data in Fig. 4 by dividing the total heat by the total moles of O_2 . The result, $-580 \text{ kJ/mole of } O_2$, is in reasonable agreement with that expected from the overall coupled metabolic reaction in the oxidation of sucrose in the nutrient media to CO_2 and H_2O (about $-500 \text{ kJ/mole } O_2$) (Lehninger, 1971). The differences between these two values may be accounted for partly by the inability to quantitate precisely ΔH of oxidation in complex biological systems, and partly by the term M in equation 1 which may be thought of as a maintenance energy term.

Taken together, our observations on the metabolic properties of plant tissues and cultured cells present a pattern consistent with growth properties of the intact plants. For example, the 38 °C and 32 °C temperature limits for reversibility of metabolic rates correspond to the critical temperatures for the heat shock responses in tomatoes (Williams, personal communication) and carrots (Choi and Sung, personal

communication; Pitto *et al.*, 1983). Tomatoes grow rapidly at high temperatures but cease growth as well as functions such as fruit ripening, flowering and seed set below 10 °C (Lyons, Graham & Raison, 1979). Carrots do not flourish at higher temperatures but continue growth at temperatures well below those where tomato growth stops. Each of these properties is correlated with results from thermal studies of the cell cultures. In addition, other phenomena such as the reversibility of metabolic shut-down at both high and low temperatures, metabolic efficiencies of cells, and oxygen dependence of metabolism were illustrated by these studies.

The calorimetric methods illustrated here promise to be useful generally in determining metabolic responses of many different cell culture preparations, tissue samples, and even cuttings, to a variety of stresses. For example, characterization of genetic variants for salt tolerance, herbicide resistance, and maximum growth temperature, might be readily examined in a multivariate study in a matter of days. This method could allow the rapid selection of cultivars to match growing conditions and thus has potential applications to studies of increased crop productivity.

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