Direct Respiration of Lipids During Heat Production in the Inflorescence of *Philodendron selloum*

Abstract. *Respiration in the heat-generating, sterile florets of* Philodendron selloum *was examined by electron microscopy and carbon isotopic analysis of respired carbon dioxide. After the spathe unfolded, the florets switched from carbohydrate oxidation to lipid oxidation, which persisted during heating and for at least 2 days thereafter. The scarcity of glyoxysome-like organelles and the low catalase activity in this tissue indicate that the lipid was respired directly and not after conversion to carbohydrate by the glyoxylate shunt. Thus, lipid metabolism in this heat-generating plant tissue appears to mimic aspects of the biochemistry and physiology of heat production in some animal tissues.*

Philodendron selloum K. Koch (Araceae) is a robust perennial plant that is native to Brazil. At the time of pollination, the spathe of a ripe inflorescence unfolds for 2 days during which the florets along the spadix are exposed (*1*). During the two nights when the spathe is open, the inflorescence reaches temperatures of up to 46°C (even in ambient air temperatures as low as 4°C), with most of the heat being generated by the sterile male florets (*2*). We report that the heating sterile florets use lipid directly and exclusively as the respiratory substrate. The specimens of *P. selloum* that we studied were grown on our campus.

Before the onset of maximum heating, the cells that make up the bulk of the sterile florets contained abundant lipid reserves in spherical organelles with only small amounts of starch in the plastids (Fig. 1A). Most lipid-storing cells contained few vacuoles. All had conspicuous mitochondria and thick cell walls. During the maximum heating in the tissues (Fig. 1B), the lipid reserves were progressively depleted, as indicated by a loss of osmiophilic contents from the cells. As heating progressed, the vacuoles containing the remaining lipid increased in size and number. During maximum heating, the mitochondria often contained enlarged cristae. Starch disappeared from the plastids before the first night of heat production. No degradation of the cell wall occurred, and few microbodies (glyoxysomes or peroxisomes) were observed.

On the basis of these observations of the ultrastructure and the observation that the respiratory quotient of the sterile florets during heating was 0.82 (*3*, *4*), we postulated that heat production in *P. selloum* might involve a switch from carbohydrate to lipid oxidation. We therefore determined the stable carbon isotope ratios of CO_2 respired by the sterile florets at various times during the thermogenic cycle. We expected that CO_2 respired by *P. selloum* during carbohydrate oxidation would have higher $^{13}C/^{12}C$ ratios than that produced by lipid oxidation because carbohydrates typically have greater $^{13}C/^{12}C$ ratios than lipids (*5*) and because the $\delta^{13}C$ values (*6*) of respired CO_2 are similar (usually 1 to 2 per mil more positive) to those of the respiratory substrates (*7*, *8*). Measurements of $\delta^{13}C$ values of respired CO_2 have been used to track the shift from carbohydrate to lipid oxidation in aging potato tissue (*8*) and in mice in which an alloxan-induced diabetic condition decreased carbohydrate oxidation and increased lipid oxidation (*9*). Thus, we expected to observe a shift toward more negative $\delta^{13}C$ values for respired CO_2 if lipid oxidation increased once the sterile florets entered the heating stage.

Results of our analysis of respired CO_2 carbon isotope ratios (Fig. 2) show that during the preheating stage the average $\delta^{13}C$ value was -22.6 ± 0.8 ($\pm$ standard deviation) per mil ($N = 7$). In contrast, during the 2 days when the spathe was open, the average value was -29.3 ± 1.5 per mil ($N = 17$). During the periods of maximum heating, the average $\delta^{13}C$ value was -29.4 ± 1.6 per mil ($N = 10$). The average $\delta^{13}C$ value of CO_2 respired by the sterile male florets for the 2 days after the spathe closed (and heating ended) averaged -28.6 ± 1.0 per mil ($N = 9$).

The data indicate that there was a shift toward more negative $\delta^{13}C$ values for CO_2 respired by the sterile male florets once the spathe opened and heating began (Fig. 2). This observation is consistent with the postulated shift from carbohydrate oxidation in the preheating stage to lipid oxidation during the heating stage. The results also suggest that the shift occurred sometime before maxi-

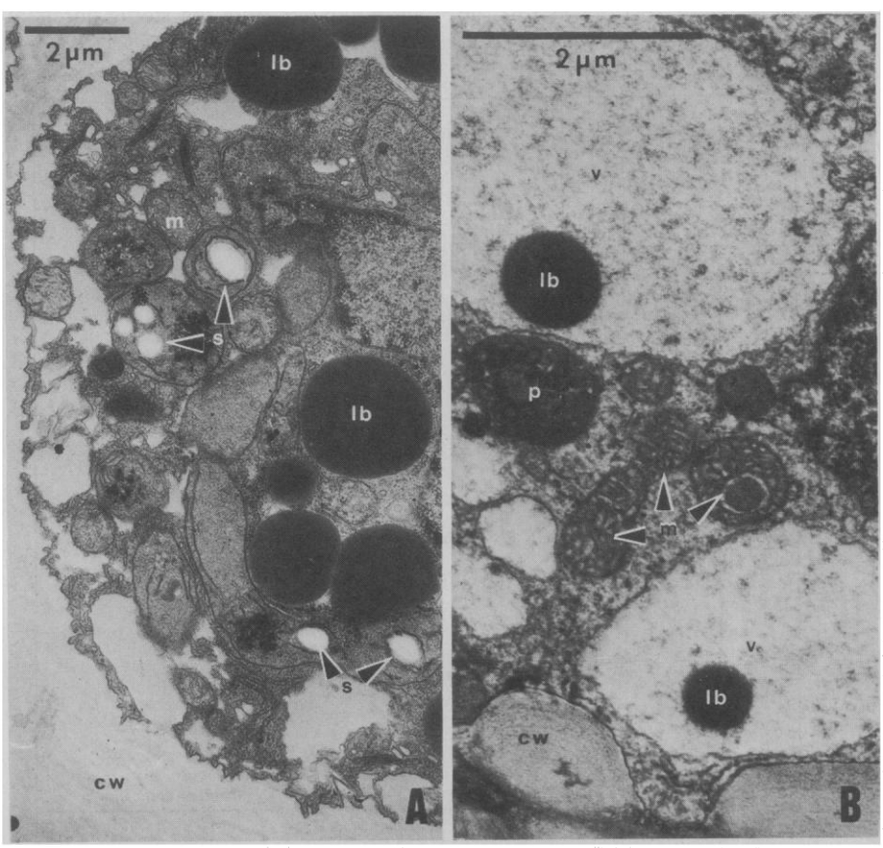

Fig. 1. Transmission electron micrographs of lipid-containing cells in the sterile florets of *Philodendron selloum*. Tissue was fixed in glutaraldehyde and osmium tetroxide and stained with uranyl acetate and lead citrate. (A) Cell before heating contains large lipid bodies and small deposits of starch in the plastids (×6800). (B) Cell during maximum heat generation contains smaller lipid bodies within expanded vacuoles, no starch in the plastids, and mitochondria with dilated cristae (×15,000). Abbreviations: *cw*, cell wall; *lb*, lipid body; *m*. mitochondrion; *p*, plastid; *s*, starch; and *v*, vacuole.

mum heating and that the florets continued to oxidize lipids after the heating ended and the spathe closed.

To strengthen the basis for interpreting the $\delta^{13}C$ values of respired CO_2, we determined the $\delta^{13}C$ values for starch and lipids during various stages of the thermogenic sequence (Fig. 2). For starch from the stalk in the region where the sterile florets were attached (*10*) the average value was -24.3 ± 1.2 per mil ($N = 6$) and that for the lipid fraction isolated from sterile florets was -29.7 ± 2.3 per mil ($N = 9$). We observed no significant differences among the $\delta^{13}C$ values of either starch or lipid isolated from the preheating, heating, and postheating stages. Thus, our isotopic analysis showed that CO_2 respired by preheating florets had $\delta^{13}C$ values similar to those of starch, and CO_2 respired from florets that were heating (or that had already heated) had values similar to those of the lipid fraction.

Finally, we examined the possibility that disappearing lipid stores were first being converted to carbohydrate by way of the glyoxylate shunt before oxidation. To evaluate the involvement of the glyoxylate shunt in this process, we assayed for catalase (E.C. 1.11.1.6), an enzyme that is universally present in plant peroxisomes and glyoxysomes, the latter being the cellular site of the glyoxylate shunt (*11*). Results from both the oxygen electrode and spectrophotometric assays were similar, but only the latter are presented (Table 1). Negligible amounts of catalase activity were detected in extracts from the sterile florets at all stages tested. Analysis of mixed extracts from *P. selloum* and castor bean endosperm, which uses the glyoxylate shunt, indicated that no inhibition of catalase by unknown factors in *P. selloum* occurred. On the basis of these results and the scarcity of glyoxysome-like organelles visible by electron microscopy, we concluded that the glyoxylate shunt was not operating and that lipids were oxidized directly to CO_2.

Table 1. Activity of catalase, a marker enzyme for glyoxysomes, in the sterile florets of *Philodendron selloum* and castor bean (*Ricinus communis*) endosperm, a tissue in which the glyoxylate shunt is known to operate, as determined spectrophotometrically after the method of Lück (*24*).

Stage	Activity (μmoles of substrate consumed per minute per milligram of fresh weight)
Philodendron selloum	
Preheating	Not detectable
Heating	0.083
Postheating	0.067
Ricinus communis	
Not applicable	10.33
P. selloum and *R. communis*	
Heating	11.05*

*The sample contained 1 mg each from *P. selloum* and *R. communis*, but activity is expressed only per milligram of *R. communis* for the purpose of comparison.

All of these data indicated that the sterile florets used starch or other carbohydrates as the respiratory substrate up to the stage at which the spathe unfolded, whereas lipids were the primary respiratory substrate during heating as well as thereafter when heating ceased, the spathe closed, and senescence of the unpollinated inflorescence ensued. Low levels of contaminating carbohydrate respiration would be difficult to detect from $\delta^{13}C$ values of respired CO_2 during peak periods of respiration. However, carbohydrate respiration should have been detectable at the postheating stage when the respiratory rate slowed to less than that during preheating (*12*).

Direct use of lipids as the predominant or exclusive substrate for respiration in plant tissues rarely has been reported

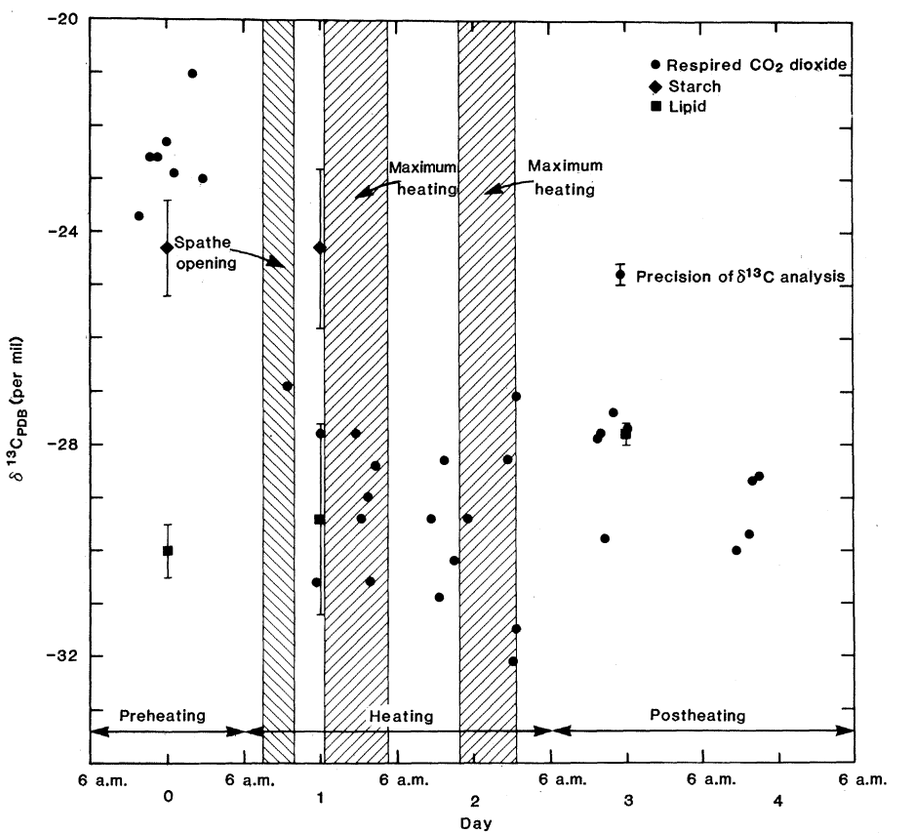

Fig. 2. The $\delta^{13}C$ values of CO_2 respired by the sterile florets of *Philodendron selloum* and of starch and lipids during the heating sequence. The 2 days of heating are indicated as days 1 and 2. Periods of maximum heating were estimated from other studies (*2, 3*). Day 0 is used for convenience to indicate preheating specimens, since it was not possible to estimate when heating would begin. The respired CO_2 was collected by passing a stream of CO_2-free air over sterile florets (dissected from freshly collected inflorescences) and then through a series of liquid nitrogen–cooled traps in a flow system similar to that described by DeNiro and Epstein (*18*). The respired CO_2 was isolated by cryogenic distillation and purified of possible trace contaminants by heating it with copper and silver at 400°C before determination of its $^{13}C/^{12}C$ ratio by mass spectrometry. Whole inflorescences were collected and stored at −70°C before extraction of the starch and lipid fractions. Sterile florets were ground in hot isopropanol with a Polytron homogenizer, and the lipid fraction extracted as described by Kates and Eberhardt (*19*). Thin-layer chromatography of the lipid fractions on precoated plates (Silica Gel 60 Merck) according to Skipski *et al.* (*20*) indicated the presence of large amounts of di- and triglycerides and fatty acids with only small quantities of phospholipids. Starch was isolated by the method of Pucher *et al.* (*21*) from the stalk of the spadices in the region where the sterile florets were attached. The $\delta^{13}C$ values were determined by combusting the starch and lipid samples by a modified version of the Stump and Frazer method (*22, 23*), purifying the CO_2 produced during combustion by cryogenic distillation, and determining the $^{13}C/^{12}C$ ratio of the CO_2 by mass spectrometry. The means and standard deviations for lipids and starch isolated from three different specimens at each stage are indicated. We were not able to isolate starch from specimens in the postheating stage. Each point for respired CO_2 represents the result of analysis from a different specimen.

but is known to occur as a temporary wound response in potato slices (*8*). In the case of heating aroid inflorescences, at least a partial involvement of lipid oxidation has been suggested in *Sauromatum* (*13*), which, unlike *P. selloum*, contains glyoxysomes (*14*). The absence of glyoxysomes or peroxisomes in *P. selloum* sterile florets suggests that lipid was being respired by mitochondrial β-oxidation, which occurs in animal tissues, rather than by glyoxysomal β-oxidation, which is common in plant tissues (*15*). The prevalence of mitochondria in the tissues of *P. selloum* is consistent with this suggestion. It has been proposed that the alternative pathway (the so-called cyanide-resistant pathway) in mitochondria is responsible for thermogenicity in aroids and other plants (*16*).

The lipid degradation that occurs during heat generation in *P. selloum* appears to be the most dramatic example of direct lipid oxidation yet reported for plant tissues. Furthermore, the use of lipids by *P. selloum* has interesting similarities to that found in animal tissues with high energy requirements, such as heart and wing muscles (*17*).

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References and Notes

1. In *P. selloum*, up to a few thousand florets are closely packed along an elongate stalk; the stalk plus attached florets is called a spadix. Three types of florets are arranged along the spadix: those producing pollen (fertile male) are located along the top, those producing heat (sterile male) are along the middle, and those producing seeds (fertile female) are along the bottom. Surrounding the spadix is a protective leaflike sheath called the spathe. Together the spathe and spadix constitute the inflorescence.
2. K. A. Nagy, D. K. Odell, R. S. Seymour, *Science* **178**, 1195 (1972).
3. R. S. Seymour, G. A. Bartholomew, M. C. Barnhart, *Planta*, in press.
4. The respiratory quotient is the ratio of the amount of CO_2 released to the amount of O_2 consumed during complete oxidation of a substrate. Complete oxidation of a carbohydrate such as starch results in equal amounts of CO_2 released for O_2 consumed, a quotient of 1.0. For lipids more O_2 is consumed than CO_2 released, and the quotient can often be as low as 0.7, depending on the lipid being oxidized.
5. R. Park and S. Epstein, *Geochim. Cosmochim. Acta* **21**, 110 (1960); *Plant Physiol.* **36**, 133 (1961); B. N. Smith, *BioScience* **22**, 226 (1972); _____ and C. R. Benedict, *Plant Cell Physiol.* **15**, 949 (1974).
6. $^{13}C/^{12}C$ ratios are expressed as $\delta^{13}C$ values, where

$$\delta^{13}C \text{ (per mil)} = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}} - 1\right] \times 1000$$

The standard is the PDB carbonate.
7. J. S. Hsu and B. N. Smith, *Plant Cell Physiol.* **13**, 689 (1972).
8. B. S. Jacobson, B. N. Smith, S. Epstein, G. G. Laties, *J. Gen. Physiol.* **55**, 1 (1970).
9. B. S. Jacobson, B. N. Smith, A. V. Jacobson, *Biochem. Biophys. Res. Commun.* **47**, 398 (1972).
10. We were unable to isolate enough starch to analyze from the sterile florets and therefore purified starch from the stalk of the spadix along the region where the sterile florets were attached. Because both the stalk and the florets are achlorophyllous tissues requiring import of all materials for growth through the vascular system, it is reasonable to assume that the $\delta^{13}C$ values for the starch accumulated in the stalk would be similar to those of starch in the adjacent sterile florets.
11. H. Beevers, in *The Biochemistry of Plants*, P. K. Stumpf, Ed. (Academic Press, New York, 1980), vol. 4, pp. 117–130.
12. The ranges and means of the respiratory rate (in micromoles of CO_2 respired per gram, wet weight, of sterile florets per minute) were 0.60 to 1.09 and 0.83 ± 0.16 for preheating specimens (N = 7), 1.27 to 7.05 and 3.67 ± 1.83 for heating specimens (N = 17), and 0.03 to 1.56 and 0.40 ± 0.53 for postheating specimens (N = 9).
13. R. H. Wilson and B. N. Smith, *Z. Pflanzenphysiol.* **65**, 124 (1971).
14. C. Berger and E. Schnepf, *Protoplasma* **69**, 237 (1970).
15. T. Galliard, in *The Biochemistry of Plants*, P. K. Stumpf, Ed. (Academic Press, New York, 1980), vol. 4, pp. 85–116.
16. B. J. D. Meeuse, *Annu. Rev. Plant Physiol.* **26**, 117 (1975).
17. R. L. Hazelwood, in *Avian Biology*, B. S. Farner, J. R. King, K. C. Parkes, Eds. (Academic Press, New York, 1972), vol. 2, pp. 471–526.
18. M. J. DeNiro and S. Epstein, *Geochim. Cosmochim. Acta* **42**, 495 (1978).
19. M. Kates and F. M. Eberhardt, *Can. J. Botany* **35**, 895 (1957).
20. V. P. Skipski, A. F. Smolowe, R. C. Sullivan, M. Barclay, *Biochim. Biophys. Acta* **106**, 386 (1965).
21. G. W. Pucher, C. S. Leavenworth, H. B. Vickery, *Anal. Chem.* **20**, 850 (1948).
22. R. K. Stump and J. W. Frazer, *Nucl. Sci. Abstr.* **28**, 746 (1973).
23. D. W. Northfelt, M. J. DeNiro, S. Epstein, *Geochim. Cosmochim. Acta* **45**, 1895 (1981).
24. H. Lück, in *Methoden der Enzymatischen Analyse*, H. U. Bergmeyer, Ed. (Verlag Chemie, Weinheim, 1962), pp. 885–894.
25. We thank G. Bartholomew, E. Gonzalez, and G. Laties for discussions and H. Ajie and D. Winter for technical assistance. Supported by a grant from the UCLA Academic Senate Committee on Research and by NSF grant ATM 79-24591.
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Primary Murine Bone Marrow Cultures Support Continuous Growth of Infectious Human Trypanosomes

Abstract. *The human parasite* Trypanosoma brucei gambiense *grew continuously at 37°C in primary cultures of murine bone marrow. Cultured parasites remained virulent for mice. Rapid parasite growth coincided with the appearance of adherent adipocyte-epitheloid cell aggregates that also promoted hematopoiesis. This culture system should permit studies of host cell control of trypanosome proliferation, pathogenic effects of trypanosomes on blood cell development, and the relative trypanocidal and marrow suppressive activities of drugs.*

The *Trypanosoma brucei* subgroup of African trypanosomes includes important pathogens of humans and domestic animals (*1*). Although these trypanosomes are usually described as blood parasites, most of them develop in the extravascular tissue space (*2*). Parasites isolated from tissue sites differ antigenically (*3*) and in reproductive activity (*4, 5*) from peripheral blood parasites. Reinvasion of the blood by tissue parasites is thought to be an important factor in relapse following drug treatment (*6*). Factors that influence the tissue distribution and physiological activity of trypanosomes in different sites are unknown. However, certain established cell lines potentiate the growth of *T. brucei* in vitro at 37°C (*7*). Interactions between trypanosomes and mammalian cells in these culture systems appear to be specific since few cell lines support parasite growth and not all parasite strains grow with the same cell line (*7*). Similar specific interactions between trypanosomes and host tissue cells could influence parasite multiplication in vivo. Bone marrow is one tissue that harbors a high proportion of dividing trypano-

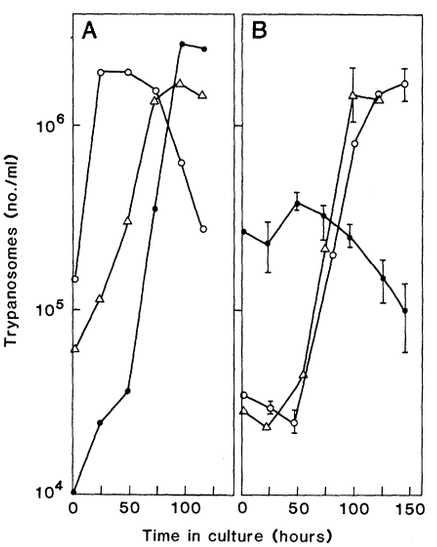

Fig. 1. Growth of *T. b. gambiense* in primary murine bone marrow cultures. (A) Marrow from BALB/cCr mice was cultured for 3 weeks and then inoculated with trypanosomes at different initial cell densities. (B) W/W^V cultures of different ages: (●), 2 weeks; (○), 3 weeks; (△), 4 weeks. The flasks were gently shaken at the times indicated, and 0.2 ml of supernatant was removed for hemacytometer counts. In (A) the values are for a single flask at each cell input. In (B) the mean and range for three flasks is shown at each culture age. All ranges are not shown to avoid obscuring mean values; variation at all time points was comparable to data shown.