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PATHWAYS OF CARBOHYDRATE OXIDATION DURING THERMOGENESIS BY THE SPADIX OF *ARUM MACULATUM*

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Summary

1. The aims of this work were to discover the pathways of carbohydrate oxidation prior to and during thermogenesis by the club of the spadix of *Arum maculatum*, and whether there was coarse control of these pathways.

2. $^{14}CO_2$ production from [1-^{14}C]-, [3,4-^{14}C]-, and [6-^{14}C]glucose, the detailed distribution of ^{14}C from [1-^{14}C]- and [6-^{14}C]glucose, and the maximum catalytic activities of phosphofructokinase, fructose-1,6-diphosphate aldolase, glucose-6-phosphate dehydrogenase, and phosphogluconate dehydrogenase were determined at different stages in the development of the spadix. The results indicate that in the early stages carbohydrate is oxidized via both the pentose phosphate pathway and glycolysis, and that a shift to glycolysis occurs during development so that just before and during thermogenesis glycolysis predominates almost exclusively.

3. During development the activities of phosphofructokinase and glucose-6-phosphate dehydrogenase per club increased 100- and 10-fold, respectively. Comparison of the activities of phosphofructokinase with the rates of carbohydrate oxidation showed that coarse control of glycolysis operates during spadix development, and indicated that the onset of rapid glycolysis at thermogenesis is regulated by fine control or availability of substrate.

Introduction

Thermogenesis, though uncommon in higher plants, occurs in a number of inflorescences. Much of our limited knowledge of this process rests on studies of the appendix of the spadix of the inflorescence of *Arum maculatum* [1]. This inflorescence passes through a number of recognizable developmental stages, which for the appendix, culminate in a sudden and rapid rise in the rate of respiration. This rise occurs at the expense of stored starch and results in heat production at a specific stage in pollination [2]. The rate of respiration

may increase 150-fold to a value [2] that so exceeds those of plant tissues in general that it is similar to those reported for hovering humming birds [3].

The first aim of our work was to assess the relative activities of glycolysis and the pentose pathway before and during thermogenesis by the appendix of the spadix of *A. maculatum*. This knowledge is important in respect of our understanding of the mechanism of thermogenesis. Our second aim was to see whether changes in the maximum catalytic activities of regulatory enzymes of glycolysis and the pentose phosphate pathway contributed to the control of carbohydrate oxidation during spadix development. In plants in general the fluxes through these pathways must, almost certainly, be regulated in the first instance by fine control [4]. However, the limits within which such fine control operates could be set by the maximum catalytic activities of the regulatory enzymes, and variation of the latter during development could be an important aspect of control where large changes in flux occur. The extent to which such coarse control operates in higher plants is not known. There is evidence from two plant tissues [5,6] that changes in the fluxes through the two pathways correlate closely with changes in the maximum catalytic activities of regulatory enzymes. However, the changes in flux were not large enough, in relation to the maximum catalytic activities, to prove that the latter contributed to the control of the former. The change in flux in *Arum* spadix is so great that we thought that this tissue would provide a more searching test of the hypothesis of coarse control of carbohydrate oxidation in plants.

We used three complementary methods to investigate the relative activities of glycolysis and the pentose phosphate pathway. These were the measurement of the patterns of $^{14}CO_2$ production from [1-^{14}C]-, [3,4-^{14}C]- and [6-^{14}C]glucose, the determination of the detailed distribution of ^{14}C after supplying [1-^{14}C]- and [6-^{14}C]glucose, and the measurement of the maximum catalytic activities of diagnostic enzymes. We chose phosphofructokinase (EC 2.7.1.11) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) because they are almost certainly regulatory, and fructose-1,6-diphosphate aldolase (EC 4.1.2.7) and phosphogluconate dehydrogenase (EC 1.1.1.44) because they are the only other enzymes which are unique to their respective pathways. We made the above measurements at different stages of development and we paid particular attention to the stages prior to and at thermogenesis. We compared the enzyme activities with estimates of the rates of carbohydrate oxidation to see if the former could regulate the latter.

Materials and Methods

Materials

[1-^{14}C]- and [6-^{14}C]glucose were obtained from the Radiochemical Centre, Amersham; [3,4-^{14}C]glucose from N.E.N. Chemicals GmbH, Dreieichenhain; substrates, cofactors and enzymes from Boehringer, Mannheim.

All our work was done with the swollen club-shaped portion of the appendix of the spadix of *A. maculatum* L. Inflorescences, at different stages of development, were taken from plants growing wild in a range of local sites during April and May in 1974 and 1975. The time between collection of inflorescences and the start of the experiments was 1—3 h. The clubs were not cut off the

spadices until the start of the experiments. We recognized five developmental stages of the inflorescence which we characterized (Table I) and refer to as α, β, γ, pre-thermogenesis and thermogenesis. The first four of these stages were recognized by the length of the inflorescence, the furling of the spathe and the weight of the club. Thermogenesis was recognized by the rate of oxygen uptake of excised but otherwise intact clubs.

Metabolism of [^{14}C]glucose

For these experiments clubs were cut transversely into slices 1 mm thick. Comparisons between differently labelled [^{14}C]glucose were made only within the same batch of replicate samples. At α stage 4—6 similar clubs were cut and the slices were randomized and divided into replicate samples of 300 mg fresh weight. At all other stages the replicate samples for an individual experiment were prepared from a single club. The fresh weight of these samples varied from 180 to 200 mg depending upon the club. Each sample of slices was put on a circle of Whatman No. 3 filter paper supported on a single layer of glass beads (diameter 2 mm) in the main compartment of a Warburg manometer flask. Each incubation was started by carefully adding the [^{14}C]glucose solution in a way that soaked the filter paper but did not immerse the slices. The [^{14}C]glucose was dissolved at 0.5 mM in 0.02 M KH_2PO_4 (pH 5.2), and 1.5, 1.0 and 0.75 ml were added to samples at α stage, pre-thermogenesis, and thermogenesis, respectively. The specific activities were 0.5 Ci/mol [1-^{14}C]- and [6-^{14}C]glucose; 0.15 Ci/mol [3,4-^{14}C]glucose. The flasks were shaken gently throughout the incubation which was at 25°C in the dark. $^{14}CO_2$ was collected in alkali in the centre well. At the end of the incubation the [^{14}C]glucose solutions were removed and each sample was given three rinses, each with 3.0 ml of 0.02 M KH_2PO_4 (pH 5.2), before being killed with boiling 80% (v/v) aq. ethanol. The tissue was extracted successively with boiling 80% (v/v) aq. ethanol. boiling 20% (v/v) aq. ethanol and boiling water. The extracts were combined, and then evaporated under reduced pressure at 28°C. The residue was extracted with ether to give the fats and then with water to give the water-soluble substances. The latter were fractionated into basic, acidic, and neutral components by ion-exchange chromatography as described by Harley and Beavers [7] except that we used 4 M formic acid to elute the acidic fraction from the Dowex 1. Sugars were isolated from the neutral components by paper chromatography [8].

Enzyme assays

For measurement of enzyme activity at α stage we used samples of four clubs; for all other stages each sample consisted of a single club. Clubs were homogenized in 4—8 volumes of extraction medium in a small pestle and mortar, and then in an all-glass homogenizer. Examination of the homogenates with a microscope revealed very few unbroken cells: For α and β stages the extraction medium was 40 mM glycylglycine buffer (pH 7.4); for the other stages it was 100 mM glycylglycine buffer (pH 7.0) which contained EDTA (20 mM), cystein—HCl (20 mM) and sodium diethyldithiocarbamate (20 mM). The extracts were centrifuged at 100 000 × g for 30 min and the supernatant fractions were assayed for enzyme activity at once. Protein in the extracts was measured by the Folin method [9].

TABLE I

CHARACTERISTICS OF DEVELOPING SPADICES

Protein was extracted as described under enzyme assays and was assayed by the Folin method. The measurements of gas exchange were made with excised but otherwise intact clubs at 25°C with a Thoday respirometer. Each value is the mean ± S.E. of measurements made on a number (shown in parentheses) of different spadices.

Characteristic	Stage of development				
	α	β	γ	Pre-thermogenesis	Thermogenesis
Length of inflorescence (cm)	8.9 ± 0.1 (27)	15.1 ± 0.3 (15)	19.2 ± 0.3 (17)	20.7 ± 0.8 (15)	—
Length of club (cm)	3.1 ± 0.1 (34)	5.3 ± 0.2 (17)	5.8 ± 0.2 (18)	5.8 ± 0.3 (16)	—
Weight of club (mg)	220 ± 10 (42)	661 ± 37 (16)	1130 ± 96 (18)	1261 ± 180 (18)	752 ± 75 (35)
Extracted protein (mg/club)	5.1 ± 0.3 (18)	19.7 ± 1.3 (16)	34.1 ± 2.9 (18)	40.3 ± 5.1 (16)	21.2 ± 1.4 (35)
Oxygen uptake (µl/h per g fresh wt.)	554 ± 10 (8)	756 (2)	—	949 ± 89 (13)	9000—30 000
Respiratory quotient	0.99 ± 0.03 (3)	0.93 (2)	—	0.95 ± 0.06 (5)	0.93 ± 0.01 (6)

All enzymes were measured at 25°C by methods described previously [6]. The reaction mixtures, final volume 3.0 ml, contained: phosphofructokinase, 6 mM fructose 6-phosphate, 0.15 mM NADH, 1 mM $MgCl_2$, 2 mM cysteine, 0.5 mM ATP, 0.14 unit aldolase, 60 μg of a mixture of glycerolphosphate dehydrogenase and triosephosphate isomerase, in 40 mM glycylglycine (pH 8.2); fructose-1,6-diphosphate aldolase, 2 mM fructose 1,6-diphosphate, 0.2 mM NADH, 15 μg of a mixture of glycerolphosphate dehydrogenase and triosephosphate isomerase, in 40 mM glycylglycine (pH 8.1); glucose-6-phosphate dehydrogenase, stages α to pre-thermogenesis, 2.5 mM glucose 6-phosphate, 0.5 mM NADP, 20 mM $MgCl_2$, 0.012 unit phosphogluconate dehydrogenase, in 50 mM glycylglycine (pH 8.9); glucose-6-phosphate dehydrogenase at thermogenesis, as above except that glucose 6-phosphate was 15 mM; phosphogluconate dehydrogenase, stages α to pre-thermogenesis, 1.0 mM 6-phosphogluconate, 1.0 mM NADP, 13 mM $MgCl_2$, in 50 mM glycylglycine (pH 8.5); phosphogluconate dehydrogenase at thermogenesis, as above except that 6-phosphogluconate was 5 mM and $MgCl_2$ was 6.5 mM.

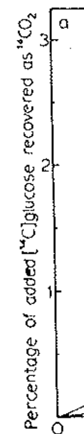
Miscellaneous

Gas exchange of whole clubs was measured on samples of single clubs lain on filter paper moistened with 0.02 M KH_2PO_4 (pH 5.2). Warburg's direct manometric method was used for clubs at α stage. At the other stages gas exchange was measured as changes in volume at constant pressure in a Thoday respirometer [10]. Respiratory quotients are calculated from measurements of oxygen uptake and CO_2 production by the same club. ^{14}C was measured by liquid scintillation spectrometry and ^{14}C in insoluble material was converted to $K_2{}^{14}CO_3$ before counting [11].

Results

Spadix development

We distinguished five stages in the development of the spadix. These stages are defined and characterized by the data in Table I. Stages α, β, and γ correspond almost exactly to similarly designated stages in previous studies [2,12, 13]. The stage which we call pre-thermogenesis is 6—18 h before thermogenesis and is characterized by being neither warm nor smelly, having a spathe that is about to unfurl, and having a relatively low rate of respiration. We designated a spadix as being at thermogenesis only if we had shown that the oxygen uptake of the excised club was greater than 9 ml/h per g fresh weight. The rates of oxygen uptake of the thermogenic clubs which we used varied but many were between 20 and 30 ml/h per g fresh weight. This variation was probably due to the fact that peak activity is maintained for only a brief period [2]. From Table I it can be seen that the rate of respiration of the clubs rose gradually from α stage to pre-thermogenesis but the extent of this increase was small compared with that which occurred at thermogenesis. Both fresh weight and protein content of the clubs rose during development up to pre-thermogenesis and then fell sharply during thermogenesis.



Metabolism of [^{14}C]glucose

In order to label clubs with [^{14}C]glucose in a reproducible way we had to use slices of clubs. We found that slicing caused an immediate 50—70% increase in the rate of respiration. The oxygen uptake of the clubs may reach values so high in relation to the diffusion coefficient of oxygen in water that suspension of slices in aqueous solutions would limit respiration. Thus we labelled the slices by laying them on filter paper moistened with [^{14}C]glucose solutions. Under these conditions the slices did not absorb a great deal of label but replicate samples agreed closely in respect of uptake, $^{14}CO_2$ production, and labelling of tissue components. We monitored oxygen uptake during the feeding experiments and showed that the rates did not fall below those expected from the data in Table I.

Characteristic patterns of $^{14}CO_2$ production at α stage, pre-thermogenesis, and thermogenesis were obtained consistently (Fig. 1). At all stages carbons 3 and 4 of glucose were released more readily than carbons 1 and 6. The crucial feature of these patterns is that at α stage the release of C-1 appreciably exceeded that from C-6, whereas at pre-thermogenesis and thermogenesis we detected no appreciable difference between the yields from C-1 and C-6. The patterns obtained at pre-thermogenesis and thermogenesis were similar except that release was more rapid at thermogenesis.

The detailed distribution of ^{14}C in samples of slices, which had been supplied with [1-^{14}C]- and [6-^{14}C]glucose is shown in Table II. The data are from samples incubated for the shortest times commensurate with adequate uptake of ^{14}C. Thus the precise conditions of feeding varied with the stage, and no significance should be attached to the differences in uptake at the different stages. Uptake by duplicate samples fed [1-^{14}C]- and [6-^{14}C]glucose was similar. Examination of these results shows that the labelling patterns were not seriously affected by

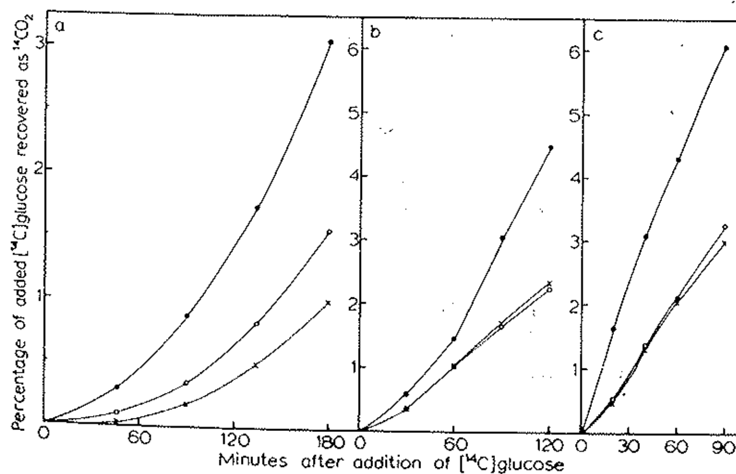


Fig. 1. Release of $^{14}CO_2$ from (○——○) [1-^{14}C]-, (●——●) [3,4-^{14}C]-, and (X——X) [6-^{14}C]glucose supplied to slices of clubs of spadices. Samples at stages α (a), pre-thermogenesis (b), and thermogenesis (c) of fresh weight 300, 180, and 185 mg were incubated in 1.5, 1.0, and 0.75 ml 0.5 mM [^{14}C]-glucose, respectively. The specific activities were 0.5 Ci/mol for [1-^{14}C]- and [6-^{14}C]glucose and 0.15 Ci/mol for [3,4-^{14}C]glucose. Each point is the mean of data from duplicate samples.

TABLE II

DISTRIBUTION OF ^{14}C RECOVERED FROM SLICES OF SPADICES SUPPLIED WITH [1-^{14}C]GLUCOSE AND [6-^{14}C]GLUCOSE

Slices of spadices were incubated in [^{14}C]glucose as described in Fig. 1. At the end of the incubations the slices were rinsed with 0.02 M KH_2PO_4 (pH 5.2), killed with boiling 80% ethanol, and extracted successively with 80% ethanol, 50% ethanol, and water. The water-soluble substances in the extracts were fractionated by ion-exchange chromatography. The ^{14}C recovered as $^{14}CO_2$, in fats, in the water-soluble substances and in the water-insoluble substances was summed to give the amount of ^{14}C absorbed by the samples.

Stage of development	Position of ^{14}C in glucose	Incubation time (min)	Percentage of added ^{14}C absorbed	Percentage of absorbed ^{14}C recovered per cell fraction					
				CO_2	Fats	Water-soluble substances			Water-insoluble substances
						Basic components	Acidic components	Neutral components	
α	1	90	15.9	2.0	0.7	3.4	3.4	78.6	8.0
	6		16.5	1.1	0.2	4.7	4.4	76.7	8.3
Pre-thermogenesis	1	120	6.6	21.6	1.4	23.4	10.1	30.7	5.4
	6		6.7	21.4	0.7	22.5	10.1	33.9	7.1
Thermogenesis	1	80	4.7	42.0	0.2	14.7	14.4	24.8	0.3
	6		5.8	38.0	0.2	16.7	14.2	26	0.7

losses during analysis. At α stage 75% of the label recovered from the tissue was in the neutral fraction. Analysis by paper chromatography showed that 67% of the label in this neutral fraction was present as [^{14}C]glucose and 28% as [^{14}C]-sucrose. This neutral fraction and the insoluble material were labelled equally by C-1 and C-6. In contrast C-1 made a greater contribution to CO_2 than did C-6, whilst C-6 made correspondingly greater contributions to the basic and acidic fractions. These differences are small in relation to the total ^{14}C reccovered from the tissue but they were obtained consistently. The differences are more obvious if the labelling of the fractions is related to the amount of [^{14}C]glucose oxidized rather than to the total ^{14}C recovered in the tissue. The sum of the ^{14}C recovered as $^{14}CO_2$, in fats, in the acidic and basic fractions, and in the insoluble material, represents the macimum amount of [^{14}C]glucose which could have entered glycolysis and the pentose phosphate pathway. If the labelling of the CO_2, and the basic and acidic fractions is expressed as a percentage of this total in each case then the values for C-1 are 11, 19 and 20, and for C-6, 6, 25 and 23. More prolonged incubation, 180 min, of α stage tissue in [^{14}C]glucose gave results similar to those in Table II except that there was less difference between the contributions of C-1 and C-6 to the basic and acidic fractions.

The distribution of label from [^{14}C]glucose at pre-thermogenesis shows that C-1 and C-6 labelled all fractions almost equally. Compared to α stage, CO_2, and the acidic and basic fractions were more heavily labelled whilst the proportion of label recovered in the neutral fraction declined. In the neutral fraction, 67% of the label was present as [^{14}C]sucrose, 10% as [^{14}C]glucose and 10% as [^{14}C]fructose. At thermogenesis C-1 and C-6 again labelled all fractions to almost the same extent. There was a sharp rise in the labelling of the CO_2 and a fall in that of most other fractions. Very little label was recovered in the insoluble material. In the neutral fraction [^{14}C]sucrose, [^{14}C]glucose, and [^{14}C]-fructose accounted for 66, 27 and 6% of the label, respectively. Longer incubations (100 min) of thermogenic tissue did not alter the relative contributions of C-1 or C-6 to any fraction.

Enzyme activities

We assayed the activities of the enzymes of glycolysis and the pentose phosphate pathway at stages α, β, γ, pre-thermogenesis and thermogenesis. We optimized the conditions for the assays of all four enzymes by varying the concentration of each component of the assay mixtures and by varying the pH. We did this at α stage and at thermogenesis. In addition, we investigated the reliability of our extraction procedures. First, we added pure enzymes to clubs in extraction medium, extracted the clubs, and measured the recovery of the pure enzymes in the extracts. In each of these experiments we halved a single club lengthwise and extracted and assayed one half in the normal way. We extracted the other half in medium which contained measured amounts of commercially purified samples of the enzymes to be assayed. Recovery of the pure enzymes was assessed by comparing the activities in the extracts of the two halves of the club. Recoveries were determined with clubs at stages α, β, γ, pre-thermogenesis and thermogenesis. With one exception the recoveries of all four enzymes at all stages of development were within 10% of the value predicted from measurements of the added activity and that present in extracts of the club alone. The

TABLE III

ACTIVITIES OF ENZYMES OF GLYCOLYSIS AND PENTOSE PHOSPHATE PATHWAY DURING DEVELOPMENT OF SPADIX

Single clubs were extracted and enzymes were assayed as described in the text. Activities are given as μmol substrate consumed/min per club. Values are means ± S.E. The number of clubs assayed is given in parentheses. Fisher's P values are given for comparison of the activities of clubs at different stages of development. Values of 0.05 or less are considered significant. Values greater than 0.05 are given as N.S. (not significant).

Enzyme	Activity				
Stage of development:	I α	II β	III γ	IV Pre-thermogenesis	V Thermogenesis
Phosphofructokinase	0.21 ± 0.02 (5)	2.10 ± 0.23 (6)	11.06 ± 1.66 (8)	22.43 ± 3.18 (9)	19.98 ± 3.12 (7)
Fructose-1,6-diphosphate aldolase	0.68 ± 0.04 (5)	4.56 ± 1.07 (7)	13.63 ± 1.75 (6)	17.34 ± 2.07 (10)	10.24 ± 1.75 (7)
Glucose-6-phosphate dehydrogenase	0.37 ± 0.01 (7)	1.96 ± 0.20 (10)	2.97 ± 0.48 (7)	3.58 ± 0.53 (6)	2.44 ± 0.19 (6)
Phosphogluconate dehydrogenase	0.76 ± 0.03 (6)	3.74 ± 0.41 (10)	6.40 ± 0.93 (7)	7.99 ± 1.20 (6)	4.51 ± 0.35 (6)

	Fisher's P values									
	I vs. II	I vs. III	I vs. IV	I vs. V	II vs. III	II vs. IV	II vs. V	III vs. IV	III vs. V	IV vs. V
Phosphofructokinase	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.05	N.S.
Fructose-1,6-diphosphate aldolase	<0.02	<0.001	<0.001	<0.01	<0.001	<0.001	<0.02	N.S.	N.S.	<0.05
Glucose-6-phosphate dehydrogenase	<0.001	<0.001	<0.001	<0.001	<0.05	<0.01	N.S.	N.S.	N.S.	N.S.
Phosphogluconate dehydrogenase	<0.001	<0.001	<0.001	<0.001	<0.02	<0.01	N.S.	N.S.	N.S.	<0.02

exception was glucose-6-phosphate dehydrogenase at pre-thermogenesis where the observed value was 19% higher than that predicted. The above procedure would give misleading results if the *Arum* enzymes were more readily inactivated than were the added enzymes which were from animals or micro-organisms. We investigated this possibility by the following type of experiment. We prepared three samples of tissue, one of α stage clubs, one of β stage clubs, and one of a mixture of equal weights of α and β stage clubs. We measured the enzyme activities in the extracts of the three samples. We found that for each enzyme the activity in the extract of the mixture was within 10% of the value predicted from the measurements made on the two separate components of the mixture. Such close agreement between the observed and predicted measurements would not be expected if inhibition or activation of the enzymes during extraction were the cause of the differences in activity which we found between the two stages. In view of the above results we suggest that our measurements of the enzymes reflect the maximum catalytic activities of the developing clubs.

The activities of the enzymes (Table III) are expressed per club because the number of cells in a club [14] changes much less than either fresh weight or protein content during development from α stage to thermogenesis (Table I). We found that the activity of phosphofructokinase increased markedly at each stage up to pre-thermogenesis but did not change at the onset of thermogenesis. Fructose-1,6-diphosphate aldolase behaved somewhat similarly except that the increase was less marked and there was evidence of a fall in activity at thermogenesis. The activities of the two dehydrogenases increased up to γ stage but not thereafter. Overall, the activities of the two dehydrogenases of the pentose phosphate pathway increased 10-fold whilst the activities of aldolase and phosphofructokinase increased 25- and 105-fold, respectively. None of the enzymes increased between pre-thermogenesis and thermogenesis. Thus during develop-

TABLE IV

COMPARISON OF ENZYME ACTIVITIES AND RATES OF CARBOHYDRATE OXIDATION

Enzymes were extracted and assayed as described in Table III. Each value is the mean of data from at least five different clubs. Rates of carbohydrate oxidation were estimated from CO_2 production which was calculated from the data in Table I; the higher value for thermogenesis was taken.

Measurement	Basis	Activity (μmol/min)			
		α stage	β stage	Pre-thermogenesis	Thermogenesis
Phosphofructokinase	per club	0.21	2.10	22.43	19.98
Glucose-6-phosphate dehydrogenase		0.37	1.96	3.58	2.44
Carbohydrate oxidation		0.04	0.16	0.39	2.38
Phosphofructokinase	per mg protein	0.041	0.155	0.664	0.854
Glucose-6-phosphate dehydrogenase		0.082	0.107	0.091	0.128
Carbohydrate oxidation		0.008	0.008	0.010	0.112
Phosphofructokinase	per g fresh weight	1.15	4.10	18.15	30.00
Glucose-6-phosphate dehydrogenase		2.24	3.08	2.68	3.97
Carbohydrate oxidation		0.19	0.24	0.31	3.17

ment there was a dramatic shift in the relative activities of the key enzymes phosphofructokinase and glucose-6-phosphate dehydrogenase towards the former. This is apparent regardless of the basis of expression (Table IV). The activities of phosphofructokinase and glucose-6-phosphate dehydrogenase were similar in α stage clubs collected at the very beginning of the season and towards the end of it. Thus the change in relative activities was associated with the development of the spadices and not with the date of their collection.

Discussion

By combination of different characteristics we were able to specify each stage of development quite accurately (Table I). Where comparable data exist, our measurements of the general properties of the spadices agree closely with published values [2,12,13]. Our results and those in the above references show that development of the club of *Arum* spadix from about α stage onwards involves cell expansion rather than cell division, and that this expansion is accompanied by marked net syntheses of protein and starch. This process is complete by pre-thermogenesis, and the onset of thermogenesis involves a sudden increase in respiration at the expense of stored starch. This increase is also accompanied by a net breakdown of protein.

At α stage the pattern of $^{14}CO_2$ production from [^{14}C]glucose is that expected from the combined operation of glycolysis and the pentose phosphate pathway. Similar results have been obtained for other plant tissues [15,16]. The pattern at pre-thermogenesis is very similar to that at thermogenesis. At neither stage is there any convincing evidence for the operation of the pentose phosphate pathway and at both stages the patterns strongly suggest an almost complete predominance of glycolysis.

The above views are supported by the detailed distribution of label from [1-^{14}C]-, and [6-^{14}C]glucose. There is strong evidence that, in plants, the ribulose 5-phosphate formed in the pentose phosphate pathway is converted to hexose phosphate and triosephosphate, most of which are subsequently metabolized to pyruvate via glycolysis [17]. Thus the combined operation of the pentose phosphate pathway and glycolysis would metabolize [^{14}C]glucose to give, initially, an excess of C-1 over C-6 in CO_2 and a corresponding excess of C-6 over C-1 in compounds derived from triose phosphate. The labelling pattern at α stage conforms to this prediction. At both pre-thermogenesis and thermogenesis all fractions were labelled almost equally by C-1 and C-6. This pattern would result if the [^{14}C]glucose were metabolized via glycolysis.

Our measurements of enzymes show that at stages α and β the activities of the glycolytic enzymes were comparable to those of the pentose phosphate pathway, and all the activities were similar to those reported for the rapidly differentiating region of pea roots [6,16]. This picture changed dramatically between β stage and pre-thermogenesis, as during this time the activities of the glycolytic enzymes increased far more than those of the pentose phosphate pathway. By pre-thermogenesis there was a very marked predominance of the glycolytic enzymes. This predominance, which exceeds by far any reported for higher plant tissues, was maintained during thermogenesis.

At each of the stages studied there is close agreement between the implica-

tions from the patterns of $^{14}CO_2$ production, the distribution of ^{14}C from [1-^{14}C]- and [6-^{14}C]glucose, and the enzyme activities. From this agreement we draw the following conclusions. First, it is unlikely that slicing the clubs caused any qualitative change in the pathways of carbohydrate oxidation. Second, at α stage both glycolysis and the pentose phosphate pathway make appreciable contributions to carbohydrate oxidation. The pattern of carbohydrate oxidation at this stage of development is very similar to that described for other developing plant tissues [5,6,16]. The general metabolism of [^{14}C]glucose at α stage shows that much is stored or used in biosynthesis and it is likely that the need for reducing power for this biosynthesis accounts for the activity of the pentose phosphate pathway. Our third conclusion is that as development proceeds the contribution of glycolysis to carbohydrate oxidation increases markedly in relation to that of the pentose phosphate pathway so that at pre-thermogenesis and thermogenesis there is an almost complete dominance of glycolysis. This dominance is consistent with our present understanding of the role of the pentose phosphate pathway in plants [17] and the mechanisms of thermogenesis [18]. Fourthly, we conclude that there is no qualitative change in the pathways of carbohydrate oxidation at thermogenesis. There was, at thermogenesis, a marked increase in the proportion of exogenous glucose which was converted to CO_2. Although this increase was at the expense of storage and biosynthesis (Table III) it is clear that thermogenesis does not preclude biosynthesis as there was appreciable synthesis of sucrose, and small but reproducible synthesis of insoluble material at thermogenesis.

Consideration of coarse control of glycolysis and the pentose phosphate pathway must centre upon the activities of phosphofructokinase and glucose-6-phosphate dehydrogenase as these almost certainly catalyse regulatory steps. Our results (Table IV) show that, regardless of the basis of expression, development of the club involved a very large increase in the activity of phosphofructokinase. Compared to this change, increases in the activity of glucose-6-phosphate dehydrogenase were small or non-existent. We do not attach great significance to the increases in enzyme activity per protein or fresh weight which occurred between pre-thermogenesis and thermogenesis. This is because both protein content and fresh weight fell at this stage. The extent to which the disproportionate increase in phosphofructokinase activity exerts coarse control over glycolysis can be judged by comparing the activity with the rate of carbohydrate oxidation during development.

The marked changes in carbohydrate make it impossible to predict the carbohydrate content of any individual spadix at thermogenesis. This precludes direct measurement of carbohydrate content as a means of measuring the rate of carbohydrate oxidation. Thus we have estimated these rates from CO_2 production, calculated from the data in Table I. The relationship between CO_2 production and carbohydrate oxidation depends upon the extent to which compounds other than hexose give rise to CO_2 and the extent to which respiratory intermediates are used in biosynthesis. The respiratory quotients of unity (Table I), the rest of the data in this paper, and the changes in carbohydrate during spadix development [2] provide very strong evidence that most, if not all, of the CO_2 produced by the clubs comes directly from carbohydrate. The data in Table II indicate that little carbon is diverted from respiratory interme-

diates at thermogenesis. Therefore at this stage we divided the rates of CO_2 production by six to obtain a minimum estimate of the rate of carbohydrate oxidation. There was diversion of respiratory intermediates into biosynthesis at the other stages of development but it is unlikely to have exceeded two-thirds of the carbon entering the respiratory pathways. Thus an estimate of the maximum rates of carbohydrate oxidation at these stages was obtained by dividing the rates of CO_2 production by two.

The above estimates of the rates of carbohydrate oxidation are compared with the activities of phosphofructokinase and glucose-6-phosphate dehydrogenase in Table IV. The following conclusions are drawn. First, at all stages examined the activity of phosphofructokinase substantially exceeded the rates of carbohydrate oxidation. This was also true of glucose-6-phosphate dehydrogenase except at thermogenesis. Thus at any specific point in development it is unlikely that the maximum catalytic activities of these enzymes is the immediate limitation on the rate of carbohydrate oxidation. Second, phosphofructokinase activity at pre-thermogenesis is high enough to support not only the highest rates of carbohydrate oxidation which we found but also the even higher rates reported by Lance [2]. This fact and our observation that phosphofructokinase activity did not increase significantly at the onset of thermogenesis strongly indicate that fine control mechanisms or, conceivably, increased production of hexose phosphate are immediately responsible for the rapid increase in glycolysis at thermogenesis. Finally, the activities of phosphofructokinase at the early stages of development are insufficient to support the rates of carbohydrate oxidation observed at thermogenesis. Thus the gradual increase in the activity of phosphofructokinase which occurs during spadix development is a pre-requisite for the attainment of the high rates of glycolysis at thermogenesis. Therefore we argue that the changes in the maximum catalytic activity of phosphofructokinase which occur during development exert coarse control over carbohydrate oxidation. The mechanism which causes these changes is not apparent from our results but the simplest hypothesis is that during the protein synthesis which occurs in spadix development there is a much more extensive synthesis of glycolytic enzymes than of the enzymes of the pentose phosphate pathway. Expression of the enzyme activities on a protein basis (Table IV) provides support for this view.

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