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METABOLIC POOLS IN THE SPADIX OF *ARUM* DURING FLORAL DEVELOPMENT

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SUMMARY

The concentration of certain metabolic intermediates in the spadix of *Arum* has been determined over the complete period of floral development from the point at which the spathes are first visible in the leaf base to thermogenesis in the spadix. These intermediates include sucrose, glucose, fructose, pyruvate, alanine, citrate, α-ketoglutarate, glutamate, glutamine, malate, aspartate and asparagine. Assays for these intermediates were supplemented by measurements of mitochondrial respiratory activity, soluble and protein-bound flavins, thiamine and ethanol-insoluble materials.

These constituents and processes reveal four broad patterns of change indicating a complex system controlling floral development and thermogenesis. The clearest indication of a specific control seen is the correlation of low mitochondrial respiratory activity with low levels of soluble flavins, suggesting that lack of these cofactors limits the respiratory activity of young spadices.

INTRODUCTION

Attention has been focused on the maturation of the spadix of *Arum* inflorescence primarily because of the dramatic shift in metabolism which is epitomized by the onset of thermogenesis. This developmental process is grossly characterized by a rise in temperature of the spadix accompanied by a change from the use of the cyanide-inhibitable cytochrome oxidase as the terminal oxidase for the mitochondrial electron transport system to a system in which the primary flow of electrons from oxidation of the substrates of the tricarboxylic acid cycle is by an alternate, cyanide resistant, pathway which includes an as yet unidentified terminal electron acceptor. This pathway is operationally defined by the resistance of respiration to cyanide or other cytochrome oxidase inhibitors and by its sensitivity to hydroxamic acids and other heavy metal chelators.

The shift of oxidation from the normal electron transport pathway which is coupled with the synthesis of ATP to the alternate pathway which is not coupled, results in the appearance of part of the energy of oxidized substrates as heat and the consequent rise in temperature of the tissue which is observed (Meeuse, 1975). The means by which this shift is controlled at the mitochondrial level has been extensively sought and attributed to a variety of factors (Hackett, 1959; Bendall and Bonner, 1971; Meeuse, 1975; Solomos, 1977).

Another pronounced shift which occurs during spadix maturation is the change from a

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relatively low level of respiratory activity (whether measured in intact tissues or in isolated mitochondria) to a very high rate of respiration which again is found in both intact cells and in isolated mitochondria (James and Beevers, 1950; Simon, 1957; Bendall and Bonner, 1971; Wedding *et al.*, 1973a, 1973b). The specific respiratory activity of *Arum* spadix mitochondria at late stages of spadix development ranks among the highest observed with plant mitochondria (Lance, 1974). It is sometimes assumed that the change from very low to very high respiratory rates simply reflects the release of the moderating influence of coupling sites 2 and 3 on the overall oxidative processes which occurs when the alternate pathway is activated. There are, however, many observations which are not in accord with this generalization. Early in the developmental process, for example, the complete blocking of cytochrome oxidase by cyanide often results in an increased oxidative rate, both in spadix slices and in isolated mitochondrial preparations (Wedding *et al.*, 1973a, 1973b, 1975, 1976). On the other hand, in the late stages of spadix development when extraordinarily high rates of respiration are observed, tissue slices or mitochondria are often significantly sensitive to cyanide, and rates may be increased by uncouplers. There is also evidence that at least part of the limitation on respiratory activity in *Arum* arises from control of enzymes whose reaction sequence preceeds the electron transport system and coupled phosphorylation. It has been shown (Wedding *et al.*, 1975) that mitochondria of young spadices have high concentrations of oxaloacetate unless extensively washed, and that the stimulation of oxidation in such mitochondria by the addition of cyanide is due to the removal of oxaloacetate by cyanohydrin formation. Although in this case accumulation of a metabolic intermediate, oxaloacetate, can be shown to be limiting the respiratory activity of the spadix, the question of what regulation may have brought about such an accumulation of oxaloacetate (the product of a reaction whose equilibrium normally lies far to the left) remains unresolved. It does not seem probable that the explanation lies in the presence or absence of an active alternate electron transport pathway.

As those who work with aroid spadix metabolism quickly discover, these tissues are highly variable in their behaviour. Genetic variability, conditions of growth (temperature, moisture, nutrients etc.), and conditions of handling after harvest (temperature, length of storage, mechanical damage) all are capable of influencing results and producing data which appear to be in direct contradiction to established dogma concerning any aspect of spadix behaviour.

Although it is clear that the processes which are involved in spadix development are intricate and complex, the primary focus of research on this tissue has been on the fact of thermogenesis and on the events which immediately preceed it, e.g. activation of the alternate pathway, etc. (Meeuse, 1975). Relatively little information on shifts in patterns of metabolism which may underlie and influence thermogenesis has been obtained. An exception is the recent work of ap Rees *et al.*, 1976 who have studied the changes in carbohydrate metabolism and in the activity of certain enzymes of glycolysis and the pentose phosphate pathway during spadix development in *Arum maculatum.*

The present report is of studies which represent an attempt to follow changing patterns of metabolism by determining the content of certain key intermediates in *Arum* spadix over the period from the first appearance of the floral structure until the onset of thermogenesis results in senescence and death of the tissue.

MATERIALS AND METHODS

Since a variety of environmental and other factors influence the characteristics of *Arum*

spadix metabolism (Wedding *et al.*, 1975) any attempt to evaluate the changes which are intrinsic to the process of floral development must of necessity involve the testing of observed differences by statistical procedures. To help minimize these factors, we have sampled an *Arum* population growing in the wild near Oxford in two successive years by two different procedures. In the spring of 1976 samples of the spadices from six to twelve plants representing stages of growth from the point of penetration of the spathe tip through its sheathing leaf base to that at which floral parts began to wither were collected within a period of 2 h at two neighbouring locations. During the 1977 season similar stages of development were collected over a period of about 4 weeks. The stage which was typical of the average spadix development of the population was collected. The 1977 samples were taken from four locations, including the two used in 1976. In both years the samples were classified according to developmental stage as defined by James and Beevers (1950) except that an attempt was made on the basis of appearance and other characteristics to select intermediates between their α-γ categories, as well as groups which clearly met the criteria of the primary categories. Thus in both years spadices were collected in nine categories rather than the five used by James and Beevers. Their final stage ζ, where spadices are withered and nearing death, was not included in this study. In most cases each category was represented by duplicate samples.

Inflorescences picked between 08.00 and 10.00 hours were brought into the laboratory, the spadices removed and immediately preserved with boiling 50% ethanol. The preserved samples were shipped by air to California where they were stored at -76°C until prepared for analysis.

Spadices cut into 1-cm lengths were chilled in an ice bath and homogenized twice for 30-s periods with a 10-min cooling period between, using a Polytron homogenizer. In 1976 homogenization took place immediately after collection and killing, in 1977 the spadices were homogenized just prior to analysis. Aliquots of the homogenate were centrifuged for 20 min at 27,000 ***g***. Each pellet was rinsed with redistilled water equal to 1/2 the volume of the original aliquot in 1976, or 1/4 the original volume of 50% ethanol in 1977. The original supernatant volume and the rinse were combined.

Enzymatic analyses of most intermediates were carried out by standard methods, in 1.0 ml volumes, using a 10-mm light path in a Gilson model 2400 automatic sample changing spectrophotometer. Except where noted, assays were as described by Bergmeyer, Garwehn and Grassl (1974). Enzymes were obtained from Boehringer Mannheim Biochemicals or from Sigma Chemical Co. and were all of grades prepared for analytical purposes. Sufficient levels of enzymes were used to bring assays to completion within 30 min, and total absorbance changes were held within the range of 0.05-0.9 Å. A known solution of the intermediate being assayed was added at the completion of assays to assess quantitation.

Glucose, fructose and sucrose were assayed in sequence in the same cuvette, using glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), hexokinase (E.C. 2.7.1.1), phosphohexose isomerase (E.C. 5.3.1.9) and fructofuranosidase (E.C. 3.2.1.26). TES buffer, pH 7.6, was substituted for triethanolamine buffer. Pyruvate was assayed with lactate dehydrogenase (E.C. 1.1.1.27), using TES buffer at pH 7.6 rather than phosphate buffer at pH 7.5. Alanine dehydrogenase (E.C. 1.4.1.1) was used to measure alanine. Citrate was assayed using citrate lyase (E.C. 4.1.3.6). Levels of α-ketoglutarate were determined with glutamate dehydrogenase (E.C. 1.4.1.3). The same enzyme, suspended in 50% glycerol instead of ammonium sulphate, was used to measure glutamate in glycine plus hydrazine buffer at pH 9.0 (Bernt and Bergmeyer, 1963). Glutamine was measured by conversion to glutamate with glutaminase (E.C. 3.5.1.2) according to the procedure recommended by Sigma Chemical Co., and a

small aliquot assayed for glutamate. Malate was determined using malate dehydrogenase (E.C. 1.1.1.37) according to the method of Hohorst (1963). Aspartate was measured using glutamate oxaloacetate transaminase (E.C. 2.6.1.1) and malate dehydrogenase. Asparaginase (E.C. 3.5.1.1) was added to the same assay for determination of asparagine. The reaction was complete within 30 min, and the secondary reaction of asparaginase with glutamine was not apparent.

Thiamine was assayed by conversion to thiochrome with alkaline ferricyanide (Airth and Foerster, 1970). Fluorescence was measured in a Turner Model 210 spectrofluorometer. The excitation wavelength was 360 nm. Emission was monitored between 400 and 650 nm, with measurements made at the maximum at 440 nm.

Flavins were also assayed fluorometrically (Cerletti and Giordano, 1971) using the same instrument. The excitation was at 435 nm, and emission was monitored between 500 and 650 nm, with the maximum at 535 nm being used for measurement. Protein-bound flavins were obtained by the procedures described, from the 50% ethanol precipitate.

After analyses were complete it was apparent that the intermediate categories did not significantly differ from the broader categories, e.g. $\alpha-$ and $\alpha+$ were very similar to α, although often different from β. The extra groups were therefore treated as representatives of the broader categories, thus providing additional replication.

The quantities of intermediates obtained from the enzymatic or other assays were expressed as concentrations per kg fresh weight tissue and the results were analyzed statistically by means of a two-way analysis of variance which allowed separate estimation of the variance associated with the two sampling periods, the stage of development and replicates. For most, although not all, of the intermediates, a significant difference between sampling years was found. In no case was there a significant difference between replicates. The variance associated with stage of development was used to estimate the differences between stages using Duncan's "*D*" test (Snedecor and Cochran, 1967). The significance of differences from maximum values at $P < 0.05$ are shown in the Figures by use of lower case letters.

The large variability of the *Arum* population which arises both from genetic and micro-environmental factors makes it difficult to demonstrate the statistical significance of any but very large differences in the characteristics of spadices at different stages of development. In the data which follow, each metabolic intermediate was assayed on all samples from a particular year at the same time and every effort was made to reduce analytical variation to a minimum. Replicate determinations on the same samples indicate that the standard error of the analytical procedures was in the range of 5-15%, some assays being more easily replicated than others. The assay results were analysed in a two-way analysis of variance with the major categories being stage of development (α, β, γ, δ, ϵ) and the sub-categories being years (1976, 1977) with replications provided in each category by multiple samples of spadices at the same stage of development. As indicated above, these replicates include a fairly wide range of developmental stage. Each analysis of variance for a given intermediate gave estimates of significance for differences between developmental stage and between years. In most cases the F value for differences between years was significant and in all cases was larger than that for differences between developmental stages. In the figures presented here the bars represent mean values for all samples at a given stage for both years and statistical significance is indicated by lower case letters associated with each histogram bar. Values having the same letter are statistically part of the same population, those having different letters are different from the maximum value at $P < 0.05$. Because of the large differences required for significance (30-70% in most cases) we tend to interpret a

situation in which a continuing trend occurs in three or more successive stages as representing a real trend of metabolite concentration with development if the differences at the extremes of the trend are significant.

RESULTS

Sugars. Concentrations of the sugars sucrose, glucose and fructose found in *Arum* spadix during development of the flowers from stage α to stage ϵ are shown in Fig. 1. In every sample but one, sucrose is present at a concentration lower than that of either of its constituent molecules, glucose and fructose. The overall mean sucrose concentration, 10.4 mM, is significantly lower than either glucose or fructose at $P < 0.05$ as shown by the t-test. During spadix growth the content of sucrose continually diminishes, although only at stages δ and ϵ is the difference statistically significant.

Glucose and fructose, on the other hand, show approximately uniform concentrations up to the end of the development period, but the ϵ spadices have significantly lower concentrations of both sugars. Over the entire period the mean concentration of glucose is 30.1 mM, half again as large as that of fructose (20.8 mM) and a t-test shows the difference is significant at $P < 0.01$.

Attempts to assay for hexose phosphates revealed that none were present. This is probably due to the method used to kill and preserve the tissues for analysis.

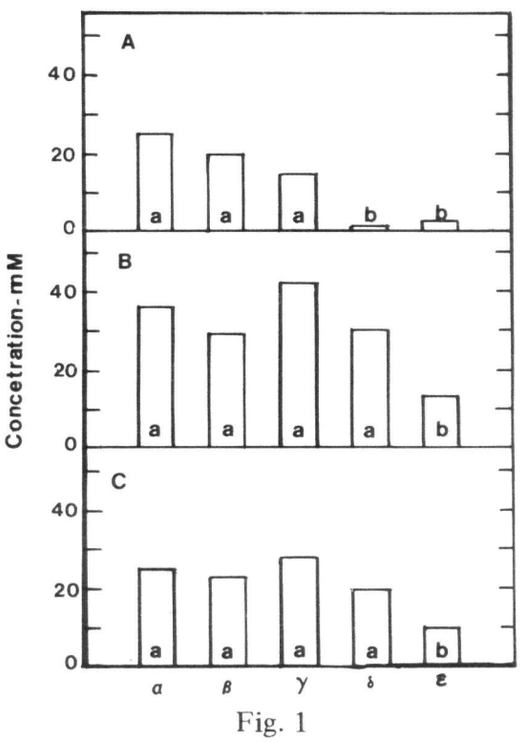

Fig. 1

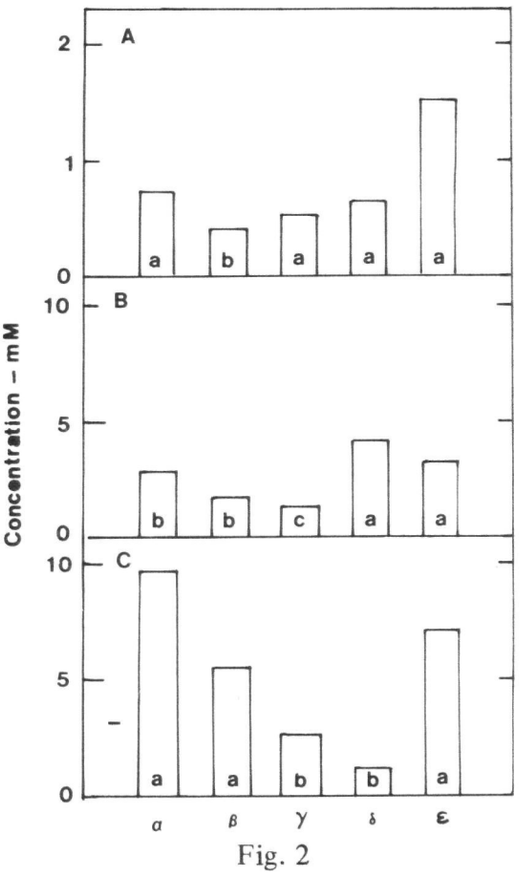

Fig. 2

Fig. 1. Concentration of sucrose, glucose and fructose in spadix of *Arum* during floral development from stage α to stage ϵ. Concentrations are are expressed as quantity per kg spadix fresh weight. Histogram bars associated with different letters are significantly different from highest value at $P < 0.05$. A = Sucrose; B = glucose; C = fructose.

Fig. 2. Concentration of pyruvate, alanine and citrate in spadix of *Arum* during floral development. Statistical significance as in Fig. 1. A = Pyruvate; B = alanine; C = citrate.

Pyruvate, alanine and citrate. The concentrations of pyruvate, alanine and citrate, substances which might be assumed to be related to one another because of their metabolic proximity, are displayed in Fig. 2. Pyruvate (which under the conditions used probably includes oxaloacetate, found to be absent or barely detectable) appears to drop in concentration during the intermediate stages of development and then to rise to its highest value in the oldest spadices at stage ϵ. Alanine, which is in metabolic communication with pyruvate through transaminases and alanine dehydrogenase, shows a similar trend, as also does citrate, an indirect product of pyruvate breakdown through acetyl CoA participation in the citrate synthase reaction. The highest concentrations of citrate are found in the youngest spadices, with an intermediate decrease followed by a rise at stage ϵ to a level which appears somewhat lower than the initial value.

The mean concentrations of these three metabolic intermediates over the full cycle of floral development are: pyruvate, 0.76 mM; alanine, 2.6 mM; and citrate, 5.2 mM. In this case *t*-tests reveal that both alanine and citrate are significantly higher than pyruvate at $P < 0.05$, but the difference between alanine and citrate is not significant.

α-Ketoglutarate, glutamate and glutamine. In Fig. 3 are presented the concentrations of α-ketoglutarate, glutamate and glutamine found during spadix development. These three metabolites, which are interrelated by the sequential reactions catalysed by glutamate dehydrogenase and glutamine synthetase, reveal few statistically significant differences over the season. There appears to be a general tendency for α-ketoglutarate to increase in concentration during spadix development, although only stage γ happens to be significantly different from the others. Glutamate has a somewhat inverse relationship with α-ketoglutarate but

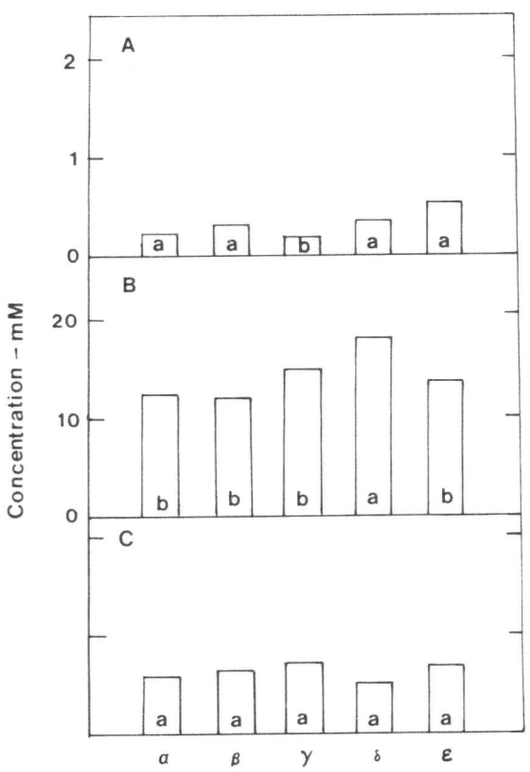

Fig. 3

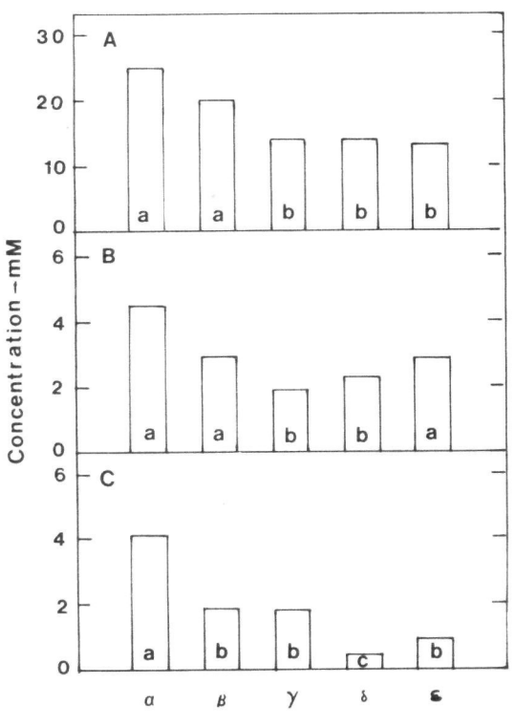

Fig. 4

Fig. 3. Concentration of α-ketoglutarate, glutamate and glutamine in spadix of *Arum* during floral development. Statistical significance as in Fig. 1. A = α-ketoglutarate; B = glutamate; C = glutamine.

Fig. 4. Concentration of malate, aspartate and asparagine in spadix of *Arum* during floral development. Statistical significance as in Fig. 1. A = Malate; B = aspartate; C = asparagine.

only stage δ is significantly higher than the other stages. Glutamine is essentially stable over the entire period, and no significant differences were found.

The differences between the mean concentrations of these three compounds over the entire floral development period is striking. α-Ketoglutarate, at 0.34 mM, is much lower than either glutamate at 14.3 mM or glutamine at 6.3 mM. All of these values are significantly different by the t-test at $P < 0.01$. These results seem to indicate that in *Arum* α-ketoglutarate is an active metabolite at all stages of development with a relatively low steady state concentration, while glutamate and glutamine may be out of the mainstream of respiratory activity and perhaps serve somewhat as storage reserves. If this is so, however, it would appear that in the seasons tested, the spadix reserves of this type have not been depleted even during the most rapid phases of growth and metabolism.

Malate, aspartate and asparagine. The concentrations of malate, aspartate and asparagine during spadix development are shown in Fig. 4. Aspartate and asparagine are in contact with the malate pool through oxaloacetate by transamination, or by way of fumarate through the activity of aspartate ammonia lyase. Aspartate and asparagine are interconnected through the asparaginase reaction. The more indirect connexion of aspartate and asparagine with malate as compared with the α-ketoglutarate, glutamate and glutamine group may be reflected in the relative concentrations of malate and the two amino compounds. Malate, at 17.4 mM, is significantly higher ($P < 0.010$) than either aspartate (2.9 mM) or asparagine (1.9 mM) when the concentrations over the entire period are averaged. Aspartate and asparagine are not significantly different. It is interesting that the relative concentrations of citrate and malate, with malate 3.3 times as high as citrate, appear to agree with the proportions of these intermediates formed when *Arum* mitochondria oxidize α-ketoglutarate (James and Elliott, 1955).

In spite of the less direct routes for interconversion of these metabolites, they display similar relative changes during development. All three start at a high level in stage α and malate and asparagine progressively diminish as the flower matures. In the case of asparagine there is a minimal concentration at the δ stage, with a significant increase as the spadix matures further.

Attempts to assay for fumarate, oxaloacetate, isocitrate, fructose-1,6-diphosphate, dihydroxyacetone phosphate, phosphoenolpyruvate and ADP showed these materials were present in concentrations too low to detect. In isolated *Arum* mitochondria James and Elliott (1955) did find detectable levels of fumarate produced from α-ketoglutarate.

Mitochondrial respiration. The level of respiratory activity measured as oxygen uptake in μmol min^{-1} mg protein^{-1} using α-ketoglutarate as a substrate is shown for stages α-ε in Fig. 5. These measurements were only from the 1976 season, in which spadices of 2-3 stages were gathered, mitochondria prepared and measurements made on the same day. Other stages were gathered on other days, and each value represents at least three replicate determinations made on different days. The results are in agreement with previous observations (Wedding *et al.*, 1975) of the respiration of *Arum* spadix mitochondria made both simultaneously and over a more extended period. This pattern is for a very low level of activity at the α stage which increases progressively until at stage ε the rate is more than ten times the rate at stage α.

It should be pointed out that the changes in respiratory activity shown in Fig. 5 are not entirely in agreement with measurements of endogenous spadix respiration (Wedding *et al.*,

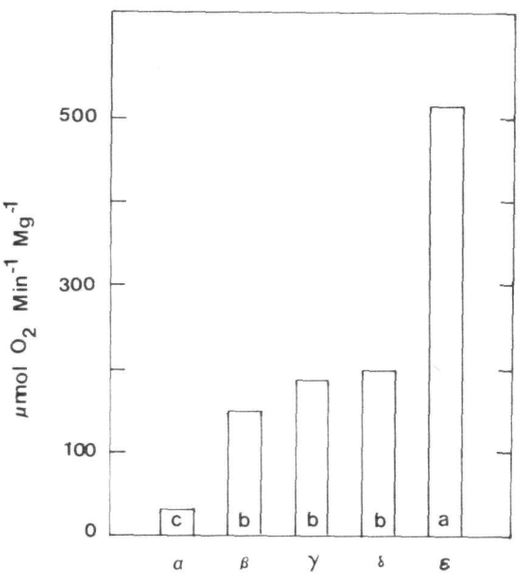

Fig. 5.

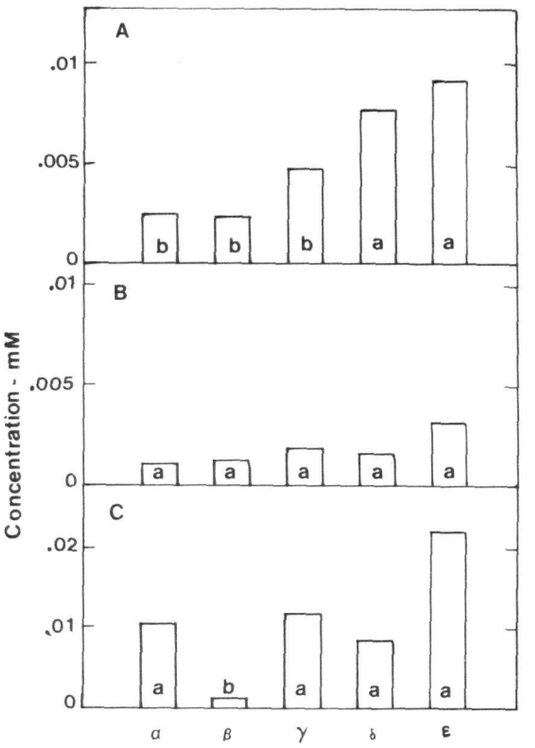

Fig. 6

Fig. 5. Changes in the rate of oxidation of α-ketoglutarate by mitochondria isolated from *Arum* spadices at different stages of floral development. Statistical significance as in Fig. 1.

Fig. 6. Concentration of flavins and thiamine in spadix of *Arum* during floral development. Statistical significance as in Fig. 1. A = Soluble flavins; B = protein-bound flavins; C = thiamine (measured as thiochrome).

1973b) where stage α spadices are reported to have a higher activity on a slice area basis with a decrease in activity at intermediate stages prior to the striking increase at the late stages of maturation. The difference may reflect, in part, differences in measurements of respiration of spadix slices using endogenous substrates as opposed to measuring activity of isolated mitochondria with substrate supplied exogenously, but probably most important is the different base on which the rates are calculated. Young spadices, with a small slice surface area, in fact display a lower rate of respiration than intermediate stages on a per slice or per spadix basis, but the smaller divisor gives a higher rate. The QO_2 values reported by James and Beevers (1950) and by Lance (1974) more nearly resemble the pattern shown in Fig. 5 except that Lance finds a maximum at stage δ both with intact spadices and with isolated mitochondria. In our view the respiratory activity of isolated mitochondria expressed on a mitochondrial protein base may be a better indicator of the respiratory capacity of the spadix at different developmental stages than endogenous respiratory rates found with intact tissues or slices.

Of the metabolic intermediates measured in these studies only citrate, α-ketoglutarate and malate are directly in the path of the TCA cycle, malate and citrate display what might intuitively be expected of TCA cycle intermediates in a situation like that shown in Fig. 5, that is, a high concentration at the time of low respiratory activity with diminishing concentrations as the activity increases. α-ketoglutarate is present as might be expected in much lower amounts than the other two acids; nor does it vary much. The slight changes in the small pool are probably of little significance in the process of spadix maturation.

What explanation can be adduced for differences in mitochondrial respiratory capacity

so marked as those shown in Fig. 5? A clue is provided by the fact that mitochondria prepared from stage *a* spadices can respond with a several-fold increase in rate to addition of exogenous flavin mononucleotide. Indeed, mitochondria from pre-stage *a* spadices (gathered at the point where the leaf base must be removed to reveal the spathe) are capable of strikingly high rates of oxidation (100-200 μmol min^{-1} mg^{-1}) when only FMN is supplied. In such a case the twice-washed mitochondria must be utilizing endogenous substrates not removed during preparation.

These observations suggest that respiratory activity may be limited in the spadix by lack of flavins. Evidence that this may be so is presented in Fig. 6. Although it would appear that about one-quarter of the flavin compounds in the spadic are bound to proteins, this fraction is likely to be less important as an explanation for restrictions on *Arum* respiratory activity than the ethanol soluble forms of these compounds. As may be seen in Fig. 6A, the concentration of soluble flavins closely parallels the relative mitochondrial respiratory activity up to stage δ. This probably indicates that the additional surge of activity found in stage ϵ is a response to some change other than increased flavin cofactors for respiratory reactions. It is likely that the major cause is to be sought in the activation of the uncoupled alternate pathway. Although there is a tendency for protein-bound flavins also to increase with maturation, the differences are not significant in these experiments.

The concentration of another respiratory cofactor, thiamine, is shown in Fig. 6C, and appears to be relatively uniform, with only stage ϵ showing a significantly lower concentration of this material.

Spadix size and weight. The data on concentrations of various metabolic intermediates in *Arum* spadices at different stages of maturity presented above are all expressed as concentrations of the various compounds calculated on the assumption that the total fresh weight of the spadix is a solvent for the intermediates. This of course establishes a minimum concentration, the actual concentration in the tissue, even if no compartmentation occurs, would be higher than this by the ratio of the fresh weight to the fresh weight minus the dry weight. Unfortunately, the sampling procedure used did not permit obtaining dry weights of the samples assayed.

Another way of expressing the content of the various intermediates would be on the basis of an amount, e.g. μmol, per spadix. This base has been used by ap Rees, 1976 in study of metabolites in *Arum* spadix. Although we feel that a concentration basis is more meaningful, we present in Table 1 the information necessary to convert our concentration figures to a per spadix base if desired.

Table 1. *Changes in the fresh weight and ethanol-insoluble residue of Arum spadix tissue at various stages of floral development*

Figures on grams per spadix followed by different letters are significantly different at $P < 0.05$

Stage	Fresh weight (grams per spadix)	Spadices per gram	50% ethanol-insoluble (%)
α	0.243 d	4.12	45.2
β	0.457 c	2.19	34.9
γ	0.859 b	1.16	35.7
δ	1.042 a	0.96	39.1
ϵ	0.777 b	1.28	29.0

Some other characteristics of developing spadices are also illustrated in Table 1. In terms of fresh weight per spadix there is a four-fold change from the α stage to the largest spadices at stage δ. The decrease between stage δ and stage ϵ may reflect some water loss as well as utilization of stored reserves in the thermogenic burst of rapid respiration.

While no dry weights are available for these samples, some indication of trends in the insoluble constituents of the spadix is given by the 50% ethanol-insoluble fraction of the fresh weight (probably composed mostly of starch, protein and cellulose). Here there is a pronounced drop between stages α and β, followed by a plateau until stage ϵ which exhibits a further decrease in this fraction.

DISCUSSION

The inherently high level of variability in all characteristics of *Arum* growing in its natural habitats has made it possible in these studies to discern only the very broadest patterns of changes in metabolic intermediates and related aspects of the spadix during floral development.

These patterns are basically four. (1) a decrease from a high level of intermediates in the youngest spadix to a low level at maturity. This is best exemplified by the sugars, particularly sucrose, and by malate. (2) An increase from a low point at the earliest stage collected to a maximum in the most mature spadices. This pattern is seen in its pure form only in the rate of mitochondrial oxidation and in the content of ethanol-soluble materials. (3) As a variant of pattern (1), this might be termed a concave pattern. A high level in young spadices diminishes during development up to a late (δ-ϵ) stage, when an increase is observed. Pyruvate, alanine and citrate—metabolically related compounds—all follow this pattern as do α-ketogutarate, aspartate and asparagine. (4) A deviant form of (2), the convex pattern, starts at a low level, reaches a maximum in the mid-stages of development and diminishes again at maturity. This pattern is shown by glutamate and flavin content as well as fresh weight per spadix. Although the differences are not significant, there also appears to be a tendency for glutamine as well as glucose and fructose to follow this pattern.

From the most simplistic approach, it might be assumed that two general patterns of change would occur during development of the flower. Constituents, particularly reserves, utilized by the developmental process would start high and drop as the the process continues, while those which are required by the process itself might start low and increase as development requires an increasing intensity of the activity in which these constituents partake. The materials and processes included in this study can be forced into these two patterns only with difficulty and the control mechanisms at work during *Arum* floral development are clearly more complex than can be elucidated by study of changes in the size of metabolic pools alone. It has been demonstrated by ap Rees *et al.* (1976) that the early part of floral development in *Arum* is characterized by activity of the pentose-phosphate shunt while the latter part of the process is dominated by glycolytic activity. Since our sampling procedures have not permitted detection of sugar phosphates, direct confirmation of this observation has not been possible, but the decrease in sucrose and in ethanol-insoluble materials presumably is a response to an increased phosphofructokinase activity such as these workers have noted.

A few indications of the nature of control processes at work during development are apparent from the present studies. The close correlation of low mitochondrial activity

with low levels of soluble flavins and the observation that washed mitochondria from young spadices show a substantially higher oxidative activity when supplied with exogenous FMN than when supplied with substrate indicate that the operation of the TCA cycle in early stages of development probably is not restricted by the availability of TCA cycle substrates. Carbohydrates, pyruvate, citrate and α-ketoglutarate are at high levels and the control of oxidative activity probably lies in the deficient supply of cofactors for flavin-requiring dehydrogenases and perhaps a lack of flavins in the mitochondrial electron transport system as well.

Up to the mid-stages of development the changes seen in these studies are consistent with an organ in a phase of rapid growth with increased demands for energy which are met by increased respiratory activity permitted in part by an increased supply of flavin cofactors and resulting in a decrease of reserves such as ethanol-insoluble materials, sugars, citrate and malate as well as lower levels of TCA cycle intermediates present in low concentration such as α-ketoglutarate. At some point, which appears to be roughly at stage δ, a shift in metabolism occurs for reasons which are not apparent from changes in the materials studied here. Although respiratory activity continues to increase and ethanol-insoluble and sucrose reserves continue to disappear, some intermediates, pyruvate, alanine and citrate, increase in concentration. Others, such as glutamate, glutamine, glucose and fructose decrease. This later phase probably coincides either with the preparation for or the onset of thermogenesis and a more detailed understanding of the patterns of accumulation and utilization of intermediates in this stage of development might be informative regarding the way in which the thermogenic process is initiated.

The studies reported here provide a broad outline of the metabolic circumstances which accompany development of the sterile spadix of *Arum* flowers. A study of this type, covering a broad spectrum of materials and processes, can only give hints of underlying causes and hopefully point toward specific reactions which can be investigated in greater detail in experiments designed to answer specific questions and to be less subject to the whims of nature and the intraspecific diversity of *Arum*. We hope to undertake a further and perhaps more enlightening approach to the study of control processes during spadix development involving the study in developing *Arum* flowers of the specific activity and control characteristics of certain enzymes suggested by the patterns seen here.

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