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CYANIDE SENSITIVITY OF RESPIRATION DURING AGEING OF *ARUM* SPADIX SLICES

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Summary

Slices of the sterile spadix of *Arum* may show variable responses ranging from inhibition to stimulation of respiration when exposed to cyanide. Some of the factors responsible for this variation in response were determined to be: (1) age of the spadix—more mature spadices tend to be unaffected or stimulated by cyanide as opposed to very young spadices, which are inhibited; (2) thickness of the slice made from the spadix—thin (*c.* 60 μm) slices are stimulated by 2.5 mM KCN while thick (>200 μm) slices are inhibited; (3) ageing of slices in aerated water—this response is complex, with slices *c.* 100 μm thick freshly cut from spadices of intermediate maturity being stimulated by cyanide initially. During the following 24 hours, however, the respiration of the slices is inhibited by cyanide, but from 2–5 days after slicing the spadix slices reach a plateau of stimulation where rates may be almost doubled by cyanide. This is followed by a decline after 8 days' ageing to a situation in which respiration is unaffected by cyanide.

An inhibitor of cyanide-resistant respiration, *m*-chlorobenzhydroxamic acid, strongly inhibits spadix respiration, with a K_i similar to that of KCN. The effect of this inhibitor is additive to that of cyanide and in appropriate conditions, the two inhibitors together will completely inhibit *Arum* spadix respiration.

Introduction

Although some degree of cyanide resistance in plant respiration is widespread, aroids such as *Sauromatum* (Van Herk, 1937), *Arum* (James and Beevers, 1950) and *Symplocarpus* (Hackett, 1957) have, because of the rapid respiration and the high degree of resistance to inhibition by cyanide exhibited by their spadix tissues, provided the material for the most intensive investigations of this phenomenon.

Stimulation of spadix tissue respiration by cyanide has been reported for *Arum* by Bendall (1958), but the most pronounced stimulatory responses evoked by cyanide have been observed with potato tubers (Hanes and Barker, 1931) and with mycorrhizal roots of the beech tree (Harley *et al.*, 1956; Harley and ap Rees, 1959). The purpose of the present investigation has been primarily to determine the factors involved in inhibitory or stimulatory responses to cyanide in *Arum*, and especially to ascertain whether ageing of tissue slices could affect the sensitivity of these cells to cyanide as has been reported for beech mycorrhizas. This study using tissue slices is intended to supplement the work already reported (Wedding, McCready and Harley, 1973) with *Arum* spadix mitochondria.

Materials and methods

Inflorescences of *Arum maculatum* L. were gathered fresh daily from plants growing in

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the wild on Headington Hill, Oxford. They were placed in a plastic bag, returned to the laboratory and held in a beaker of water until the spathe was removed and the spadix excised. Sections were cut, initially by hand using a razor blade, and in later experiments with a hand microtome. Sections were placed in distilled water, rinsed three times to remove starch granules and other cell debris and the respiration either determined immediately, or held as described under Results in various experiments.

Oxygen uptake was measured by means of a Clark-type oxygen electrode (Yellow Springs Instrument Co.) in a magnetically stirred chamber controlled at 25° C. The cell contained 2.0 ml of 0.01 M potassium phthalate buffer, pH 5.5. Cyanide additions were made by appropriate aliquots of KCN dissolved in unbuffered water. The addition of *m*-chlorobenzhydroxamic acid (*m*CLAM) was made by removing the solution in which endogenous or cyanide-treated rates had been determined and replacing it with appropriate volumes of a solution of *m*CLAM in water saturated at 20° C plus buffer and KCN as required. The concentration of water-saturated *m*CLAM at 20° C was determined spectrophotometrically to be 3.96 mM. This procedure was adopted because the use of ethanol as a solvent for *m*CLAM (Schonbaum *et al.*, 1971) was found unsatisfactory due to the marked response of respiration to the ethanol.

All the respiration determinations reported here were made with five spadix slices in the chamber. Slice diameter and thickness (by means of a micrometer) were determined when the slices were removed at the end of a run.

Potassium cyanide and potassium phthalate were supplied by British Drug Houses Ltd. The *m*CLAM was synthesized and kindly provided by Mr R. G. Powell of the Agricultural Research Council Unit of Developmental Botany, Cambridge.

RESULTS

The variety of respiratory responses to the application of cyanide which can be elicited from slices of *Arum* spadix is illustrated in Fig. 1 where tracings of oxygen electrode recordings are shown. In this preliminary experiment slices hand-cut with a razor blade from four spadices were randomly selected in groups of five slices for measurement of oxygen uptake. In the upper line, started about 30 minutes after the first slices were cut, the addition of 2.5 mM KCN has substantially inhibited the respiration of the slices. In the lower line, run about 2 hours later, the endogenous rate is somewhat lower than in the upper line, but the same concentration of KCN results, after about 1 minute delay, in a significant increase in the rate of oxygen uptake.

The two lines of Fig. 1 represent the extreme range of variability in response to cyanide observed with slices selected randomly from an apparently uniform—although not carefully chosen—population of spadices, but the general lack of uniformity in response to cyanide was so great that it seemed necessary to determine the sources of this variability.

At least three factors might be expected, on the basis of existing information, to contribute to this variable response: (1) the physiological age or stage of development of the spadix could be involved, since James and Beevers (1950) showed marked differences in the respiratory rate of *Arum* spadices in different stages of development, and Simon (1957) and Bendall (1958) have shown changes in the relative sensitivity of succinate and malate oxidation by *Arum* mitochondria to cyanide during spadix development and maturation; (2) differences in slice thickness might be responsible since Simon (1957) and Yocum and Hackett (1957) had shown that the respiration of aroid spadix slices in liquid medium

was limited by diffusion of oxygen—in the measurements reported in Fig. 1, although slices were selected for uniformity by visual inspection, hand slicing is likely to produce wedge-shaped and otherwise variable slices; (3) slice removal from the intact spadix and subsequent incubation in water for variable periods before measuring the respiration might in various ways alter their metabolism, and the length of time over which slices are so 'aged' could be related to a differential response to cyanide as was found with beech mycorrhizas (Harley and ap Rees, 1959).

As will be shown below, all three of these factors do appear to have an effect on the response of *Arum* spadix slice respiration to cyanide and probably each of them alone or in combination could be responsible for the variation in response to cyanide shown in Fig. 1.

Effect of stage of spadix development

The fact that spadices from inflorescences of which the spathe is nearly ready to open (stage γ according to James and Beevers (1950)) are different in their response to

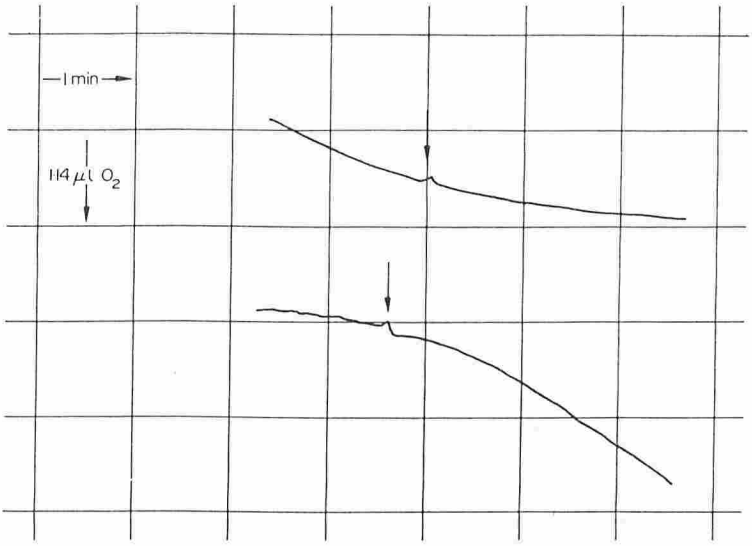

Fig. 1. Tracings of oxygen electrode recordings showing inhibition (upper line) and stimulation (lower line) of endogenous *Arum* spadix slice respiration by cyanide. Addition of 2.5 mM KCN as indicated by arrows. Tracing zero time on left.

cyanide from spadices taken from inflorescences which have only recently appeared (stage α) is illustrated in Table 1. In the experiments summarized in this table, respiratory rates and responses to cyanide were determined immediately after cutting slices. The average delay between cutting slices and final respiration measurement was about 15 minutes. Four replicates of each developmental stage were measured. These consisted of five slices cut by hand microtome and selected for uniformity of thickness from about fifty slices made of each spadix. Each replicate therefore represents a different spadix selected on the basis of morphological differences to be in stage α or γ. Preliminary experiments showed no significant difference between slices from the top and bottom of the spadix so that the five slices composing each replicate were selected randomly with respect to their location on the spadix.

The fact that spadices of stage γ selected visually are more mature than those of stage α is shown by a comparison of the diameter of spadix slices in Table 1, where it may be seen that γ spadices are approximately 1.5 times larger in diameter than those in the α stage, a highly significant difference. The rate of oxygen uptake by the more mature spadices, expressed on the basis of slice area, is about 1/2 that of the very young spadices, a difference which is also highly significant. This difference is somewhat greater than that found by James and Beevers (1950) for spadices at comparable stages of development with the rates expressed on a dry-weight basis. The last column of Table 1 shows that while slices of the young spadix are inhibited more than 20% by 2.5 mM KCN, the respiration of slices from spadices in stage γ is unaffected by the same concentration of cyanide. This difference is significant at the 5% level. It seems clear that the maturation and expansion of the spadix occurring between stage α and stage γ is accompanied by some metabolic change, expressed by a lower unit area or volume rate of respiration, which alters the sensitivity of the cells to inhibition of the transfer of electrons to oxygen via the cyanide-sensitive pathway through cytochrome oxidase.

Table 1. *Oxygen uptake and cyanide sensitivity of* Arum *spadices at different stages of maturity (respiration determinations as described in the text)*

Spadix age	Spadix diameter (mm)	Slice thickness (μm)	Respiratory rate (μl O_2 h^{-1} cm^{-2})	Plus 2.5 mM KCN (percentage of endogenous rate)
Stage α	4.6	109.6	628.2	77.7
Stage γ	7.3**	101.5	349.5**	104.5*

Statistical comparisons by *t*-test: *, significant difference with $P < 0.05$; **, significant difference with $P < 0.01$.

Effect of slice thickness

The relation of response to cyanide of *Arum* spadix respiration to the thickness of slices made from the spadix is presented in Fig. 2. These data were obtained with different lots of spadices (all approximately in stage γ) on three separate days. The data from separate days are not distinguished in the figure, but each day's samples included slices whose mean thickness covered most of the range shown. Each day slices of varying thickness were cut with a hand microtome, rinsed three times in distilled water and held in distilled water until respiratory measurements were made. Each day's set thus included slices which had been aged in water from 30 minutes to 3 hours, and the variability in response thus engendered probably contributes to the scatter of the points about the fitted lines (see Fig. 7). The slice thickness was measured after respiration measurements were complete, and the average standard error of the mean thickness of five slices was about 10% of the mean value.

In Fig. 2 the respiratory rate of the slices after addition of 2.5 mM KCN as a percentage of the endogenous rate is plotted against the reciprocal of the mean thickness of the slices. The upper line represents the response of the slices to the addition of 2.5 mM KCN alone, and the line of best fit (by the method of least squares) is: percentage of endogenous $= 60.7 + 6.91$ mm^{-1}. This line gives a correlation coefficient $r = 0.91$ with $P < 0.01$. This indicates that with spadices of about the same physiological age, measured under the same conditions, there is a highly significant change in the response to cyanide related to the thickness of the slice, with thick slices being inhibited while thinner ones are stimulated by the same concentration of KCN. An infinitely thick slice would be inhibited only 40% as indicated by the intercept on the vertical axis.

Since cyanide penetrates readily into cells in the undissociated form and since it would exist primarily in this form at pH 5.5, it seems unlikely that the differences found here are due to better penetration of the cyanide into the thinner slices. This possibility is also argued against by the fact that inhibition is more pronounced with thicker slices. It therefore seems more likely that the change in sensitivity to cyanide with a change in slice thickness is related to an alteration in the concentration of oxygen available to the cells. The rapid respiration of the *Arum* spadix is probably capable of maintaining a low O_2 tension in the intact spadix. When slices are made sufficiently thin, oxygen penetrates further into the mass of tissue and raises the effective concentration. This higher concentration of oxygen then may be a means of control, establishing the primary pathway of endogenous electron flow and making available a cyanide-resistant pathway which can accommodate the increased electron flow apparently induced by the presence of cyanide itself. Given such a stimulatory response in the presence of high oxygen concentrations, the progressive inhibition may be due to a purely physical limitation of respiration by oxygen in thicker slices, as will be discussed later.

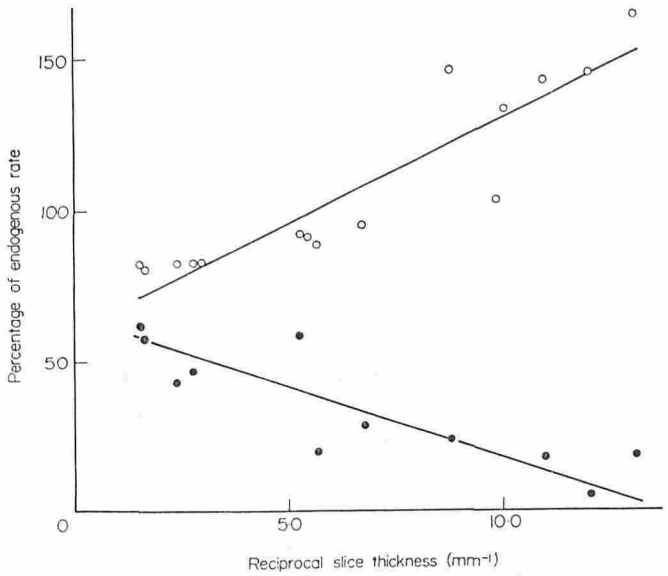

Fig. 2. Changes in the response of *Arum* spadix slice respiration to 2.5 mM KCN with changes in slice thickness. Upper line, 2.5 mM KCN alone, lower line, 2.5 mM KCN plus 3.3 mM *m*CLAM. Lines fitted by method of least squares.

The problem of oxygen limitation within the cells of an actively respiring tissue such as *Arum* spadix (Simon, 1957; Yocum and Hackett, 1957) makes it clear that depletion of oxygen within spadix cells could occur. We have compared the affinity of thin spadix slices (92 ± 15 μm) for oxygen with that of *Arum* spadix mitochondria. The slices gave a K_m for O_2 of 1.3 μM, while for the mitochondria the K_m was 0.3 μM. This fourfold difference must indicate some limitation on oxygen supply to the cells of the spadix slices under the conditions used here, and presumably thicker slices are even more deficient in oxygen supply to the cells.

The lower line of Fig. 2 shows the response of slices already treated with 2.5 mM KCN to the addition of 3.3 mM *m*CLAM. This inhibitor (see also Figs. 3 and 4) is specific for

the cyanide-resistant electron transport pathway (Schonbaum *et al.*, 1971) and in adequate concentrations in combination with cyanide would be expected to inhibit completely the respiration of *Arum* (Wedding *et al.*, 1972). The fitted line: percentage of endogenous $= 56.9 - 3.97$ mm^{-1}, $r = 0.87$ with $P < 0.05$, indicates by the intercept on the horizontal axis that such complete inhibition could be expected with slices *c.* 60 μm in thickness. Surprisingly, however, it is found that thicker slices are more resistant to the combination of *m*CLAM with KCN, and an infinitely thick slice would show no effect of the *m*CLAM addition at all. The most probable explanation for this result is that *m*CLAM penetrates into the spadix slices much less readily than does cyanide and that in very thick slices the fraction of the tissue affected by *m*CLAM would be insignificant in terms of the overall respiratory rate. In addition, the inhibition of respiration in outer layers of cells would help to raise the oxygen availability to inner cells which would otherwise be limited by oxygen.

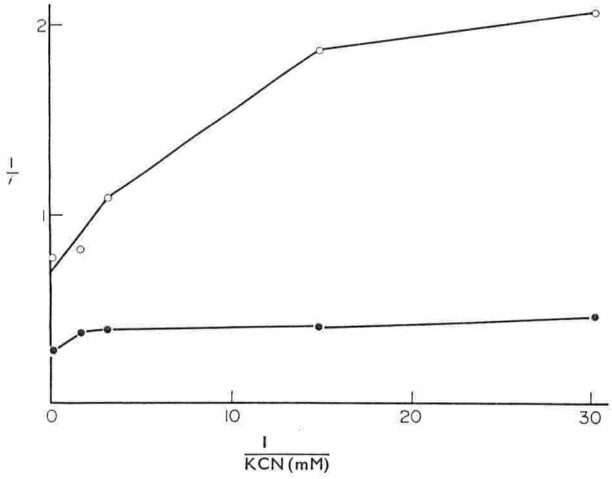

Fig. 3. Plot according to Webb (1963) showing inhibition of *Arum* spadix slice respiration as a function of cyanide concentration. Upper line, cyanide alone; lower line, cyanide plus 3.3 mM *m*CLAM.

Inhibitor constants for KCN and m*CLAM*

To determine the characteristics of the inhibition of *Arum*-slice respiration by cyanide, slices of a single spadix of stage γ were cut with a hand microtome (thickness $= 94 \pm 12$ μm), washed three times in distilled water and held 1 hour in distilled water before measurements were started. The endogenous rate was determined, followed by the addition of cyanide in concentrations ranging from 0.33 mM to 3.3 mM and after an interval sufficient to establish a rate, by the addition of 3.6 mM *m*CLAM. The results are presented in Fig. 3 as the reciprocal of the inhibited fraction plotted against the reciprocal of the cyanide concentration according to the method of Webb (1963).

A plot of this type for a simple inhibitor gives a straight line, but it may be seen from the upper line of Fig. 3 that cyanide inhibition of *Arum* spadix slices—as with *Arum* spadix mitochondria (Wedding *et al.*, 1973)—is definitely curvilinear. Although this curvilinearity makes a valid estimation of an apparent inhibition constant (K_i) for cyanide impossible, if one assumes that two different inhibition sites with different affinities for cyanide are involved, it is possible to approximate by extrapolation of the left and right ends of the curve the K_is for these two sites. These inhibition constants,

K_i = 0.0068 mM for the high affinity site and 0.15 mM for the lower affinity site (the one affected by higher concentrations of cyanide), are much more different than those obtained with *Arum* mitochondria (0.212 mM and 0.889 mM). This difference and the lower total inhibition found with the slices reflect the fact, illustrated in Figs. 1 and 2 and Table 1, that the response of intact cells to cyanide is much more complex than that of isolated mitochondria.

When *m*CLAM is added to the cyanide-inhibited slices, an additional inhibition is observed, although the curvilinear response to cyanide concentration is still found in the lower line of Fig. 3. In this case the effect of 3.3 mM KCN plus 3.6 mM *m*CLAM is an almost complete inhibition of the spadix respiration, indicating that this respiration is composed of a cyanide-sensitive fraction and a cyanide-resistant, *m*CLAM-sensitive fraction, as was found with isolated mitochondria (Wedding *et al.*, 1972).

The response of *Arum* spadix slices to *m*CLAM alone and *m*CLAM plus 3.3 mM KCN is presented in Fig. 4. Here the response of *Arum* respiration to changing concentrations of *m*CLAM produces a straight line when plotted by the same method as in Fig. 3. The

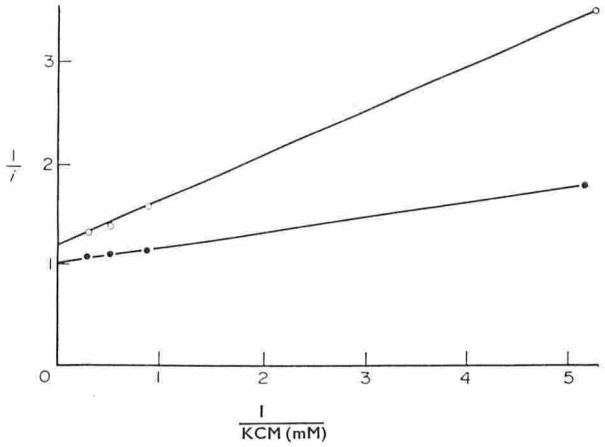

Fig. 4. Plot according to Webb (1963) showing inhibition of *Arum* spadix slice respiration as a function of *m*CLAM concentration. Upper line, *m*CLAM alone; lower line, *m*CLAM plus 2.5 mM KCN.

indicated K_i for *m*CLAM is 0.49 mM and the inhibition at infinite *m*CLAM concentration approximates 90%, indicating that in these particular slices most of the respiration was proceeding via the cyanide-resistant pathway. The addition of 3.3 mM KCN eliminates this residual fraction of respiration, giving complete inhibition at infinite *m*CLAM concentration. The lower line of Fig. 4 also shows that the addition of KCN does not alter the linearity of the response of spadix slice respiration to *m*CLAM.

Stimulation by KCN and m*CLAM of the respiration of aged* Arum *slices*

The reports that tissues resistant to cyanide such as the mycorrhizal roots of beech (Harley *et al.*, 1956; Harley and ap Rees, 1959) after several days of ageing in aerated water develop the capacity to respond to cyanide application by increased respiration raised the possibility that *Arum*-spadix slices with their substantial degree of cyanide-resistant respiration might display a similar response. In the experiments undertaken to explore this possibility, one of which is summarized in Fig. 5, slices were cut with a hand microtome from spadices primarily in the γ stage, but including small numbers of indi-

vidual spadices in the β or δ stages. The individual sets of five slices selected for respiration measurement ranged in mean thickness from 85 ± 9 μm to 107 ± 13 μm. The respiration of the first set of slices was measured starting about 1 hour after cutting. The remainder of the slices were placed in distilled water in aeration vessels (Harley and McCready, 1952) with air pulled through the sintered glass base of the vessel by vacuum. The water was changed daily, and no significant microbial growth was apparent under the microscope at the end of the ageing period. Four replicate determinations of each treatment were made on each day of sampling.

The effect of 2.5 mM KCN on the respiration of spadix slices expressed as a percentage of the endogenous rate is shown in the upper line (filled symbols) of Fig. 5. The freshly cut slices were slightly, although not significantly, inhibited by cyanide. After 24 hours cyanide caused a significant inhibition of the respiratory rate. On the second day a significant stimulation of respiration by cyanide had appeared, which persisted as a plateau until the eighth day, by which time the effect of cyanide, while still stimulatory, was significantly less than in the earlier measurements.

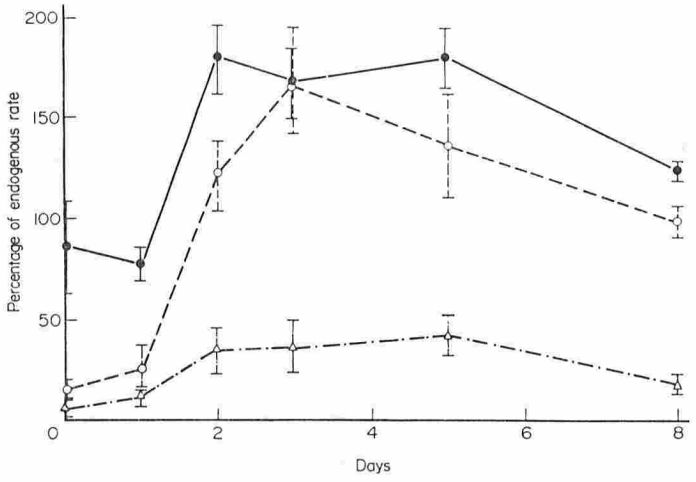

Fig. 5. Response of *Arum* spadix slice respiration to cyanide and *m*CLAM during ageing of slices in aerated water. Upper line, 2.5 mM KCN; middle line, 3.3 mM *m*CLAM; lower line, 2.5 mM KCN plus 3.3 mM *m*CLAM. Vertical bars represent standard errors of mean values.

This response of *Arum* spadix slices to cyanide is quite similar to that found with beech mycorrhizas, and since both tissues are strongly resistant to cyanide inhibition, not unexpected. More surprising, however, was the response of aged spadix slices to *m*CLAM, which is shown in the middle line of Fig. 5. In this case the respiration was strongly and significantly inhibited in fresh and 1-day-old slices, but by the end of the second day of ageing, a significant stimulation was found when 3.3 mM *m*CLAM was applied. This stimulation continued through the fifth day but on the eighth day the rate after *m*CLAM treatment was not significantly higher than the endogenous rate. The lower line of Fig. 5 represents the means of two different treatments, one in which KCN was applied first, followed by *m*CLAM and the other in which *m*CLAM was given first, followed by KCN. An analysis of variance showed that there were no significant differences between these treatments and the means of both sets are presented here. In this case it may be seen that while the total inhibition due to cyanide plus *m*CLAM does vary somewhat, with less

inhibition during the period when stimulation by KCN or *m*CLAM alone is most marked, at no time does the combination of 2.5 mM KCN and 3.3 mM *m*CLAM result in a stimulation, or indeed in less than 60% inhibition of the endogenous respiration.

The implications of these results are dealt with further in the next section, but a minimal interpretation of these data must be that in spadix slices aged 2–5 days, both the cyanide-resistant pathway, which in fresh slices of the *y* stage spadix accommodates 80–90% of the total respiration, and the cyanide-sensitive pathway are alone capable of carrying a greater total electron flow than that found in the absence of either inhibitor. This does not imply that either inhibitor increases the inherent respiratory capacity of either pathway. In Fig. 6 the rate of actual respiration of the same samples shown in Fig. 5 is given. It may be seen that the respiration of untreated slices has dropped by almost one-half after 24 hours and that this drop continues over the ageing period (filled symbols). The respiratory rate of cyanide-treated slices (open symbols) shows that even when the rate is nearly doubled by cyanide as on the third and fifth days, the rate of respiration in the presence of cyanide is only 20% or less of the initial rate of fresh slices. It is therefore

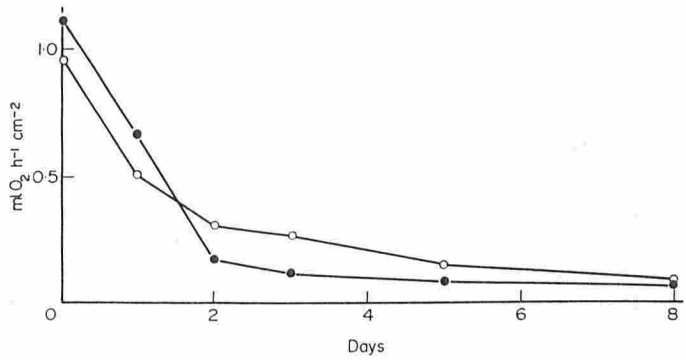

Fig. 6. Changes in the rate of endogenous respiration of *Arum* spadix slices during ageing of the slices in aerated water. Closed symbols, endogenous respiration; open symbols, plus 2.5 mM KCN.

not unreasonable to expect that sufficient capacity for electron transport would remain in either the cyanide-resistant or cyanide-sensitive pathways to provide for an increased electron flow when an inhibitor of the other pathway is given. The oxygen uptake is therefore rate limited in aged slices by some other factor than the capacity of either cyanide-resistant or cyanide-sensitive pathways.

Discussion

The response to cyanide of those plant tissues whose respiration is resistant to cyanide has long been found to be quite variable. The degree of inhibition or even the conversion of an inhibitory response to one of stimulation appears to depend on metabolic changes in the tissue or on external factors which may not be immediately obvious. In their most simple form explanations of such variability usually depend on the postulation of two alternate pathways for electron transport from the substrates of the Krebs cycle to oxygen, one of which is resistant to cyanide, while the other is readily blocked by the presence of cyanide. These hypothetical, although apparently well-founded, alternate pathways must in turn interact with associated systems, particularly that responsible for energy

conservation by phosphorylation and the primary supplier of substrate to the Krebs cycle, the glycolytic pathway. In the present studies at least three factors—spadix age, slice thickness and ageing of spadix slices—have been shown to affect in some degree the nature of the response of slices of *Arum* spadix to the application of cyanide. Due in part to the problems of working with an ephemeral tissue such as *Arum* spadix, all the information necessary to interpret these results in terms of the operation and interactions of two alternate paths of electron transport is not available, but some of the possibilities may be mentioned.

The decrease in level of inhibition by cyanide as the spadix develops and matures shown here (Table 1) has been noted before (Bendall, 1958). We have previously found (Wedding *et al.*, 1973) that *Arum* mitochondria oxidizing succinate or malate tend to be stimulated by cyanide rather than inhibited when the mitochondria are uncoupled and we have also reported some indications that mitochondria from more mature spadices are less well coupled than those from young inflorescences. It may be that the difference found here between young stage-α spadices and older stage-γ ones (James and Beevers, 1950) is due to a greater degree of uncoupling in the older tissues.

Simon (1957) found that over a period of about 1 month the cytochrome oxidase decreased markedly in *Arum* spadices from stage α to stage ε, while the succinoxidase increased more than twenty-fold in the same tissues. This is in accord with our observation that in mitochondria from mature spadices electrons from succinate appear to enter more readily the cyanide-resistant pathway and that succinate oxidation is more susceptible to stimulation by cyanide than oxidation of other Krebs cycle substrates (Wedding *et al.*, 1973).

The relationship of inhibition or stimulation by cyanide to the thickness of treated slices (Fig. 2) may be dependent on the fact that the high rates of respiration found in *Arum* spadix lead to a relative deficiency of oxygen supply to many cells even in a thin slice when oxygen is being supplied by diffusion from an aqueous medium (Yocum and Hackett, 1957). This opens the possibility of a basically physical explanation of the fact that thin slices are stimulated by cyanide while thick ones are inhibited. For such an explanation we assume that cyanide penetrates readily even into the thicker slices, but that oxygen is saturating only in a relatively thin outer layer of cells, even in the thinner slices. Cyanide is assumed to produce an increased rate of oxygen uptake via the cyanide-resistant pathway (Wedding *et al.*, 1973) in those cells where the supply of oxygen is adequate to permit this. The remainder of the cells, in spite of the presence of cyanide, would be limited by oxygen availability and would not be stimulated. As the slice is made thicker, the portion of the total tissue lacking excess oxygen becomes a larger fraction of the total. The stimulated oxygen uptake of those cells near the surface under the influence of cyanide would reduce the diffusion of oxygen into the interior of the slice by reducing the gradient, and the oxygen uptake by the interior cells would be decreased by the more limiting oxygen supply. When the slice is sufficiently thick, this reduced rate is expressed as an overall inhibition even though a small volume of cells would be respiring at a higher rate under the influence of cyanide. This explanation, of course, depends on the response of those cells adequately supplied with oxygen being one of stimulation by cyanide and we have no evidence that this may be the case other than the stimulation observed with thin slices and the response of uncoupled mitochondria to cyanide (Wedding *et al.*, 1973). Other explanations are equally possible on the basis of present evidence, including the possibility that 'wound respiration' is responsible for the stimulatory response to cyanide or that higher concentrations of oxygen are required for

the operation of the cyanide-resistant pathway than for the sensitive pathway through cytochrome oxidase. This latter possibility is consistent with the recent report (Sargent and Taylor, 1972) that a cyanide-resistant terminal oxidase in *Chlorella* has a K_m for oxygen about three times larger than that of the cyanide-sensitive oxidase, presumably cytochrome oxidase, which accounts for 65–75% of the total oxidation of these cells.

The alterations in response to cyanide which occur when *Arum* spadices are sliced and aged in aerated water are made somewhat complex by the variations in the behaviour of spadices as they develop. If spadices of intermediate maturity are sliced and the slices aged, the first stage is one in which cyanide has little effect or inhibits slightly. This is followed by a prolonged phase in which cyanide produces considerable stimulation of endogenous respiration. In Fig. 5 the latter phase is evident after 2 days ageing, but in other experiments it appeared to develop in a much shorter period of time (Fig. 7). At first sight it might seem that this stimulation could be explained by the scheme put forward for beech mycorrhizas by Harley *et al.* (1956) and Harley and ap Rees (1959).

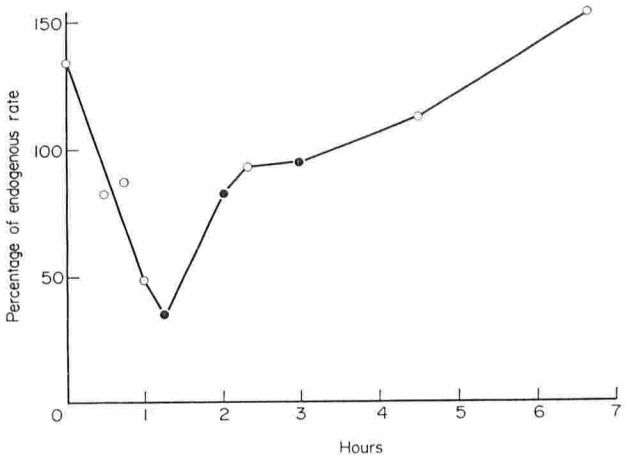

Fig. 7. Response of endogenous *Arum* slice respiration to 2.5 mM KCN as a function of time after cutting slices. Closed and open symbols represent determinations made on different days with different batches of spadices.

This explanation depends on the assumption that the glycolytic rate in the tissues becomes progressively limited by the availability of phosphate acceptors as the tissue is aged. The inhibition of the cyanide-sensitive electron-transport system, which is coupled to phosphorylation, releases this limitation and allows the cyanide-insensitive pathway to operate at an increased rate so that total oxygen uptake is increased. This explanation is, however, called into question by the stimulatory effects of *m*CLAM on the oxygen uptake of aged tissues. In this case the stimulated respiration must occur primarily through the cyanide-sensitive pathway and a stimulation of glycolysis could only be invoked if it is assumed that *m*CLAM has an additional effect of uncoupling. Such an effect of *m*CLAM is believed to be improbable (Schonbaum *et al.*, 1971; Wedding *et al.*, 1973) but little is known as yet of the possibility of other effects of this substance on metabolism. It may be that an effect on glycolysis and ADP availability need not be invoked for *Arum* slices treated with cyanide because Wedding *et al.* (1973) showed a direct stimulation of oxygen uptake in isolated mitochondria by cyanide when they were in an uncoupled state. It remains to determine the relevance of the behaviour of uncoupled mitochondria to that

of intact tissues and whether cyanide and *m*CLAM have effects on respiratory quotient and rate of carbohydrate utilization in ageing tissues.

REFERENCES

BENDALL, D. S. (1958). Cytochromes and some respiratory enzymes in mitochondria from the spadix of *Arum maculatum*. *Biochem. J.*, **70**, 381.

HACKETT, D. P. (1957). Respiratory mechanisms in the aroid spadix. *J. exp. Bot.*, **8**, 157.

HANES, C. S. & BARKER, J. (1931). The physiological action of cyanide. I. The effects of cyanide on the respiration and sugar content of the potato at 15° C. *Proc. R. Soc.*, Ser. B, **108**, 95.

HARLEY, J. L. & AP REES, T. (1959). Cytochrome oxidase in mycorrhizal and uninfected roots of *Fagus sylvatica*. *New Phytol.*, **58**, 364.

HARLEY, J. L. & MCCREADY, C. C. (1952). The uptake of phosphate by excised mycorrhizal roots of the beech. II. Distribution of phosphate between host and fungus. *New Phytol.*, **51**, 56.

HARLEY, J. L., MCCREADY, C. C., BRIERLEY, J. K. & JENNINGS, D. H. (1956). The salt respiration of excised beech mycorrhizas. II. The relationship between oxygen consumption and phosphate absorption. *New Phytol.*, **55**, 1.

JAMES, W. O. & BEEVERS, H. (1950). The respiration of *Arum* spadix. A rapid respiration, resistant to cyanide. *New Phytol.*, **49**, 353.

SARGENT, D. F. & TAYLOR, C. P. S. (1972). Terminal oxidases of *Chlorella pyrenoidosa*. *Pl. Physiol., Lancaster*, **49**, 775.

SCHONBAUM, G. R., BONNER, W. D. JR, STOREY, B. T. & BAHR, J. T. (1971). Specific inhibition of the cyanide-insensitive respiratory pathway in plant mitochondria by hydroxamic acids. *Pl. Physiol., Lancaster*, **47**, 124.

SIMON, E. W. (1957). Succinoxidase and cytochrome oxidase in mitochondria from the spadix of *Arum*. *J. exp. Bot.*, **8**, 20.

VAN HERK, A. W. H. (1937). Die chemischen Vorgänge im Sauromatum-Kolben. *Recl Trav. bot. néerl.*, **34**, 149.

WEBB, J. L. (1963). *Enzyme and Metabolic Inhibitors*, Vol. I, p. 158. Academic Press, New York.

WEDDING, R. T., MCCREADY, C. C. & HARLEY, J. L. (1973). Inhibition and stimulation of the respiration of *Arum* mitochondria by cyanide and its relation to the coupling of oxidation and phosphorylation. *New Phytol.*, **72**, 1.

YOCUM, C. S. & HACKETT, D. P. (1957). Participation of cytochromes in the respiration of the aroid spadix. *Pl. Physiol., Lancaster*, **32**, 186.